

CA1 HW 1181E67

Canada, DNHW, EHD,

81-EHD-67

A STUDY OF DISEASE INCIDENCE AND RECREATIONAL WATER
QUALITY IN THE GREAT LAKES. PHASE 1. 1981.

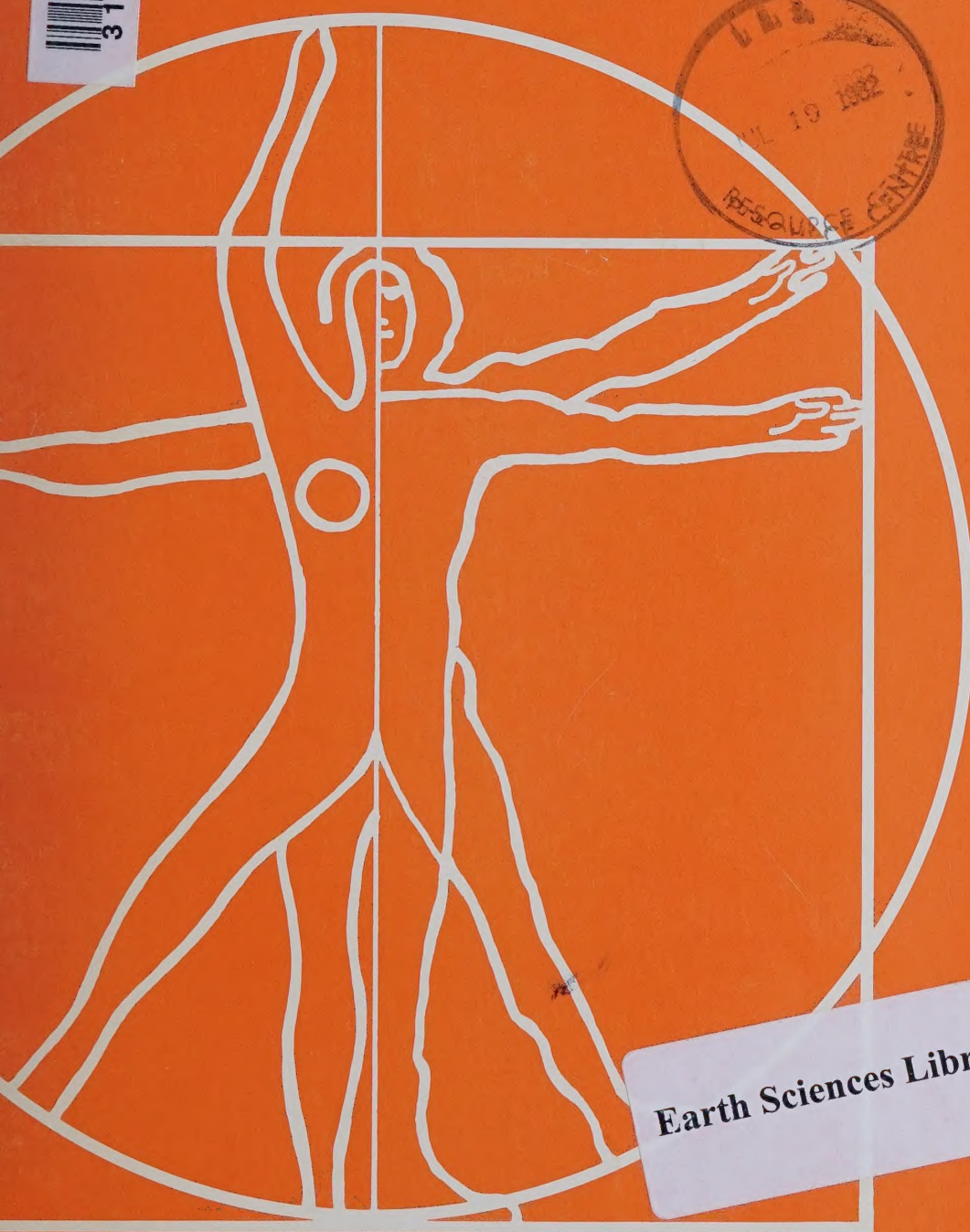
CA1 HW1181 E67

santé et Bien-être social
Canada

A study of disease incidence and recreational water quality in the great lakes phase I



3 1761 05688790 4



Earth Sciences Library



Digitized by the Internet Archive
in 2025 with funding from
University of Toronto

<https://archive.org/details/31761056887904>

February, 1982

81-EHD-67 (English)

ERRATA SHEET

1. Page 42, Table 8. Confederation Park, Sunday August 19: Pseudomonas aeruginosa was <2/100 mL water and 8/100 gm sediment. Staphylococci were 100/100 mL water.
2. Page 45, Table 8. Hanlans Point, Sunday August 19: Potentially pathogenic were +ve.
3. Page 46, Table 9. Rondeau Beach, Thursday August 23: Fecal coliforms were <2/100 mL water.
4. Page 48, Table 10. Wasaga Beach 1₃ and 2, Saturday September 8: Heterotrophic plate counts were 1.57×10^3 / mL water and 1.29×10^5 / gm sediment. Wasaga Beach 5, Saturday August 11 and Sunday August 26: Pseudomonas aeruginosa was <2/100 mL water and <2/100 gm sediment.
5. Page 51, Table 11. The eighth column should read, "Fecal coliforms in sediment /100 gm".
6. Page 132. The first paragraph should read as follows:

"Using aseptic technique, 0.2 mL of the sediment supernatant (see Preparation of Sediment for Analysis Section) is inoculated into each of 5 tubes of single strength lactosebroth (5 mL). 2.0 mL of the supernatant is inoculated into a set of 5 tubes. 20.0 mL of the supernatant is inoculated into a set of 5 double strength lactose broth tubes (10 mL). All 15 tubes are then vortexed and incubated for 24 and 48 hrs at $35 \pm 0.5^\circ\text{C}$. Those tubes showing gas fermentation are recorded as positive".

A STUDY OF DISEASE INCIDENCE AND
RECREATIONAL WATER QUALITY IN THE GREAT LAKES
PHASE I

EARTH SCIENCES
LIBRARY



Environmental Health Directorate
Health Protection Branch

Published by Authority of the Minister of National Health and Welfare

September 1980

COPIES OF THIS PUBLICATION MAY BE OBTAINED FROM:

Information Directorate
Department of National Health and Welfare
Brooke Claxton Building
OTTAWA K1A 0L2

Également disponible en français sous le titre "Étude de l'incidence des maladies
et de la qualité des eaux utilisées à des fins récréatives dans les grands lacs
Phase I"

THIS DOCUMENT WAS PREPARED UNDER CONTRACT BY:

**"THE UNIVERSITY OF TORONTO
MARCH, 1980"**

THE CONCLUSIONS AND RECOMMENDATIONS CONTAINED HEREIN REPRESENTS THE OPINION OF THE CONTRACTOR AND DO NOT NECESSARILY CONSTITUTE ENDORSEMENT BY THE DEPARTMENT OF NATIONAL HEALTH AND WELFARE

SUMMARY

The first year of a two-year study of the relationship between illness rates associated with bathing and recreational water quality was completed. This phase of the study was designed to examine potential sites for the full-scale epidemiological study, develop interviewing and microbiological techniques, and to obtain preliminary illness rate data.

Microbiological surveys were carried out at 12 beaches on Lake Ontario, 10 beaches on Lake Erie and 7 beaches on Lake Huron. Water and sediment samples were collected at the beach sites and analyzed for total heterotrophs, fecal coliforms, fecal streptococci, staphylococci (including coagulase positive Staphylococcus aureus), and Pseudomonas aeruginosa. In addition, samples were taken for the determination of pathogenic Naegleria and Acanthamoeba.

Results of the bacteriological studies showed a distinct difference in the water quality of the beaches examined. For example, Port Dover and Selkirk beaches on Lake Erie had much higher levels of bacterial indicators than Turkey Point and Rondeau Park. The St. Catharines beaches on Lake Ontario tended to show higher levels of pollution than the Toronto Island beaches. Also, Grand Bend and Ipperwash had the highest levels of fecal indicators of any of the Lake Huron beaches.

Virological analyses of lake water and sediment samples revealed no evidence of virus at the following Lake Ontario beaches: Jones, Confederation Park, Lakeside Park as well as Hanlans Point and Breakwater Beach on Toronto Island. Likewise, no viruses were found at Ipperwash, Grand Bend and the Wasaga 1, 2 and 5 beaches on Lake Huron, and the Iroquois Beach on Lake Erie. It should be noted, however, that the samples were collected in late September and October at a time when there were no swimmers present.

Water samples collected at Holiday Beach (Windsor) on September 10 and at Municipal (Weller) Park, (St. Catharines) on September 9 were positive for Naegleria fowleri. The N. fowleri isolates were deemed to be pathogenic from results of the mouse inhalation tests.

Results of the interviews showed that there was an 18.6% illness rate among the 479 swimmers at the selected beaches. The illness rate for the 39 nonswimmers was 12.8%. Because of the low number of nonswimmers, the differential is not significant. These preliminary results, however, indicate the likelihood of finding positive results in the full-scale study.

Among the swimmers surveyed, sunburn and eye, ear, and nose ailments were the most common complaints, followed by respiratory and gastrointestinal types of illnesses. Non-swimmers tended to suffer more from gastrointestinal and respiratory symptoms.

A separate study was conducted at Ipperwash Cadet Camp on Lake Huron. In this instance water and sediment samples were monitored for bacterial and viral indicators and the medical histories of the 1700 cadets at the camp during July and August were recorded. Medical records of the cadets at Ipperwash Camp revealed that upper respiratory tract infections far out-numbered gastrointestinal complaints. The incidence of boils and other staphylococcal infections as well as ear infections, possibly caused by Pseudomonas aeruginosa was also high. Future studies could be carried out at Ipperwash to demonstrate similarities between the serotypes and/or phage types of the organisms found in the water and those isolated from corresponding infections.

On the basis of these studies, several logical choices for the location of the 1980 major epidemiological study are discussed. Final choice of the locations will be made after further sampling in the spring and summer.

From this pre-trial survey it was learned that certain criteria should be considered in a study of this nature. For example:

1. Interviews are best performed on warm, sunny days when the water temperature is over 19°C.
2. If the water is rough, higher counts can be expected in the surface water samples.
3. On a sunny day, samples should be taken before noon and after 3 p.m. to eliminate the effect of U.V. light on surface water counts.
4. Both surface water and sediment analyses are necessary for a complete assessment of microbiological quality.
5. Care must be taken to ensure that there is a sufficient nonswimming population at the beach; and
6. A minimum of 2000 to 2500 swimmers and 2000 to 2500 nonswimmers should be surveyed at each beach for adequate statistical analyses.

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY.....	iv
INTRODUCTION.....	1
MATERIALS AND METHODS.....	3
BEACH LOCATIONS	3
SAMPLE COLLECTION.....	3
MICROBIOLOGICAL ANALYSES.....	3
EPIDEMIOLOGICAL SURVEY (QUESTIONNAIRES)	11
IPPERWASH CADET CAMP STUDY	12
RESULTS	29
BEACH LOCATIONS (ENVIRONMENTAL DATA).....	29
PRELIMINARY STUDIES ON MICROBIAL ENUMERATION PROCEDURES	29
MICROBIOLOGICAL ANALYSES.....	41
EPIDEMIOLOGICAL SURVEY	50
IPPERWASH CADET CAMP SURVEY.....	60
DISCUSSION	61
STATISTICAL ANALYSIS OF SURVEY POPULATIONS.....	65
DISCUSSION OF PROPOSED FUTURE SAMPLING SITES.....	68
REFERENCES.....	71
APPENDIX A - PATHOGENIC AMOEBAE	73
APPENDIX B - VIRUSES.....	83
APPENDIX C - DESCRIPTION OF BEACH SAMPLING SITES	107
APPENDIX D - MICROBIOLOGICAL METHODS	121
APPENDIX E - MEDIA PREPARATION.....	143
ACKNOWLEDGEMENTS	159
MEMBERS OF THE ADVISORY GROUP TO THE EPIDEMIOLOGICAL STUDY OF GREAT LAKES BEACHES	161

LIST OF TABLES

	<u>Page</u>
1. Sampling Dates for Lake Ontario Beaches	8
2. Sampling Dates for Lake Erie Beaches	9
3. Sampling Dates for Lake Huron Beaches.....	9
4. Interviewing Dates	27
5. Environmental Data for Lake Ontario Beaches, 1979	30
6. Environmental Data for Lake Erie Beaches, 1979	36
7. Environmental Data for Lake Huron Beaches, 1979	38
8. Microbiological Data for Lake Ontario Beaches, 1979	42
9. Microbiological Data for Lake Erie Beaches, 1979	46
10. Microbiological Data for Lake Huron Beaches, 1979.....	48
11. Geometric Means of fecal coliforms at the beaches sampled	51
12. Geometric Means of fecal streptococci, staphylococci and <u>Pseudomonas aeruginosa</u> at the beaches sampled	52
13. Order of Beach Pollution	53
14. Illness Rates for Swimmers at the Ten Beaches.....	55
15. Symptom Rates for Swimmers at the Ten Beaches	56
16. Illness Rates for Nonswimmers at the Ten Beaches	57
17. Symptom Rates for Nonswimmers at the Ten Beaches.....	58
18. Incidence of various types of illness among cadets at Ipperwash Camp	60
19. Size of groups to detect differences between two populations	66

LIST OF FIGURES

1. Location of 12 sampling sites on Lake Ontario, 10 sampling sites on Lake Erie and 7 sampling sites on Lake Huron and Georgian Bay	4
2. Sampling Sites - Hamilton and St. Catharines Region.....	5
3. Sampling Sites - Toronto Island Park	6
4. Sampling Sites - Wasaga Beach Provincial Park.....	7
5. Relative Location of Cadet Camp and Provincial Park	28

INTRODUCTION

The relationship of microbial indicators to health effects at bathing beaches is not yet clearly understood. Use of the coliform organism as water quality indicators has long dominated the thinking of sanitary bacteriologists with the result that most guidelines specify maximum permissible densities of fecal coliforms and/or total coliforms⁽²⁰⁾. It is obvious that high total counts of organisms derived from the nose, mouth, and skin would imply the direct danger of transmission of potential pathogens of many types. However, at the present time, there is not a sufficient data base for the development of microbiological criteria for recreational waters using these organisms. Furthermore, epidemiological data is lacking to show conclusively that a threshold concentration of indicator organisms exists beyond which health problems of all swimming-related diseases may be expected to increase.

To date only four epidemiological-microbiological studies have been conducted^(4, 12, 13, 19). In the most recent investigation, Cabelli and co-workers⁽⁴⁾ were able to demonstrate a good agreement between the mean densities of Escherichia coli and fecal streptococci and the differential rate of gastrointestinal symptoms in swimmers as compared with nonswimmers. As an aid for future work, Cabelli et al.⁽⁵⁾ have carefully detailed the experimental design required for epidemiological studies of this nature.

As yet there have been no studies carried out in Canada to determine the incidence of illness related to swimming in the Great Lakes. While the potential pathogens Staphylococcus aureus⁽¹⁶⁾, Candida albicans⁽¹⁸⁾, Salmonella⁽⁶⁾ and Pseudomonas aeruginosa⁽¹⁶⁾ have been shown to occur in Great Lakes waters, little attention has been given to the possibility of these organisms acting as etiological agents in swimming-related illnesses. In addition, information concerning the relationship between incidence of gastrointestinal symptoms and levels of fecal organisms is sparse.

The purpose of the present study was to gain some additional insight into the question of the potential health hazards associated with swimming at Canadian beaches on the Great Lakes. The primary objectives of Phase I of the investigation were as follows:

1. To analyze a variety of sampling locations on the Great Lakes and select sites suitable for future intensive studies. The beaches would be assessed for microbial quality, bather loads, physical ease of sampling, and public response to surveys.
2. To establish appropriate methods for the isolation and enumeration of bacterial indicators, Naegleria, Acanthamoeba, and enteric viruses from water and sediment at the beach sites.

3. To conduct surveys to determine the illness rate by interviewing bathers and non-bathers at selected beaches with a subsequent telephone or mail follow-up. The format of the questionnaires used would vary and these would be assessed at the conclusion of this step.
4. To determine if there is any correlation between rate of illness and levels of bacterial, viral, and amoebic indicators at the beach sites.

MATERIALS AND METHODS

BEACH LOCATIONS

A variety of beaches on Lakes Ontario, Erie and Huron (Figure 1) were chosen for this initial phase of the study to obtain background information on water quality and incidence of swimmers. Of the 12 Lake Ontario beaches, eight were located in the Hamilton and St. Catharines region (Figure 2), three sampling locations were at beaches on the Toronto Islands (Figure 3), and one site, Darlington Provincial Park, was located east of Toronto. There were ten sampling sites along the north shore of Lake Erie as indicated in Figure 1. Three beaches were sampled on the west side of Lake Huron and four on Georgian Bay. The three sampling sites at Wasaga Beach, on Georgian Bay, are detailed in Figure 4.

The beaches along with their appropriate sampling dates, are listed in Tables 1, 2, and 3. A detailed description of each beach site is given in Appendix C.

SAMPLE COLLECTION

Samples were collected at the portions of the beaches that had maximum bather loads. The depth of the water at each sampling site was approximately 50 cm.

- (a) Surface Water Samples were collected by holding a sterile sample bottle just under the surface of the water. The water temperature was determined by placing a thermometer in the bottle at the time of sampling.
- (b) Sediment Samples were obtained using a grab method. Wide-mouthed metal containers were run along the surface of the Lake bed to collect the top 3 cm of sediment.

Both the surface water and sediment samples were chilled during transport to the laboratory. Water samples were processed within 6 hours of collection, as well as the sediment samples.

MICROBIOLOGICAL ANALYSES

All surface water and sediment samples were analyzed for the following parameters: fecal coliforms, fecal streptococci, heterotrophic plate count, Pseudomonas aeruginosa, staphylococci (including coagulase positive Staphylococcus aureus), potentially pathogenic amoebae, and enteric viruses.

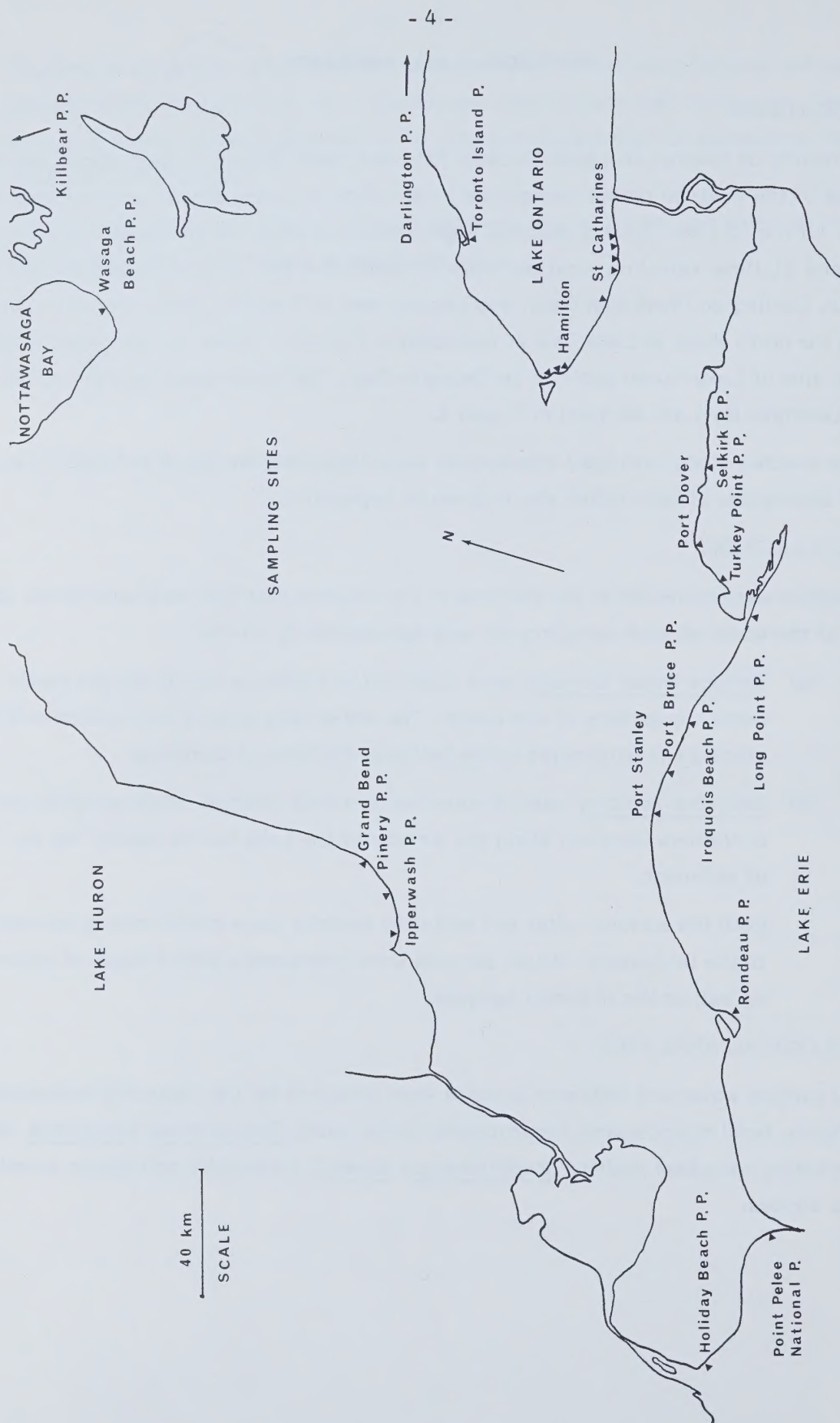


Figure 1 - Location of 12 sampling sites on Lake Ontario, 10 sampling sites on Lake Erie, and 7 sampling sites on Lake Huron and Georgian Bay

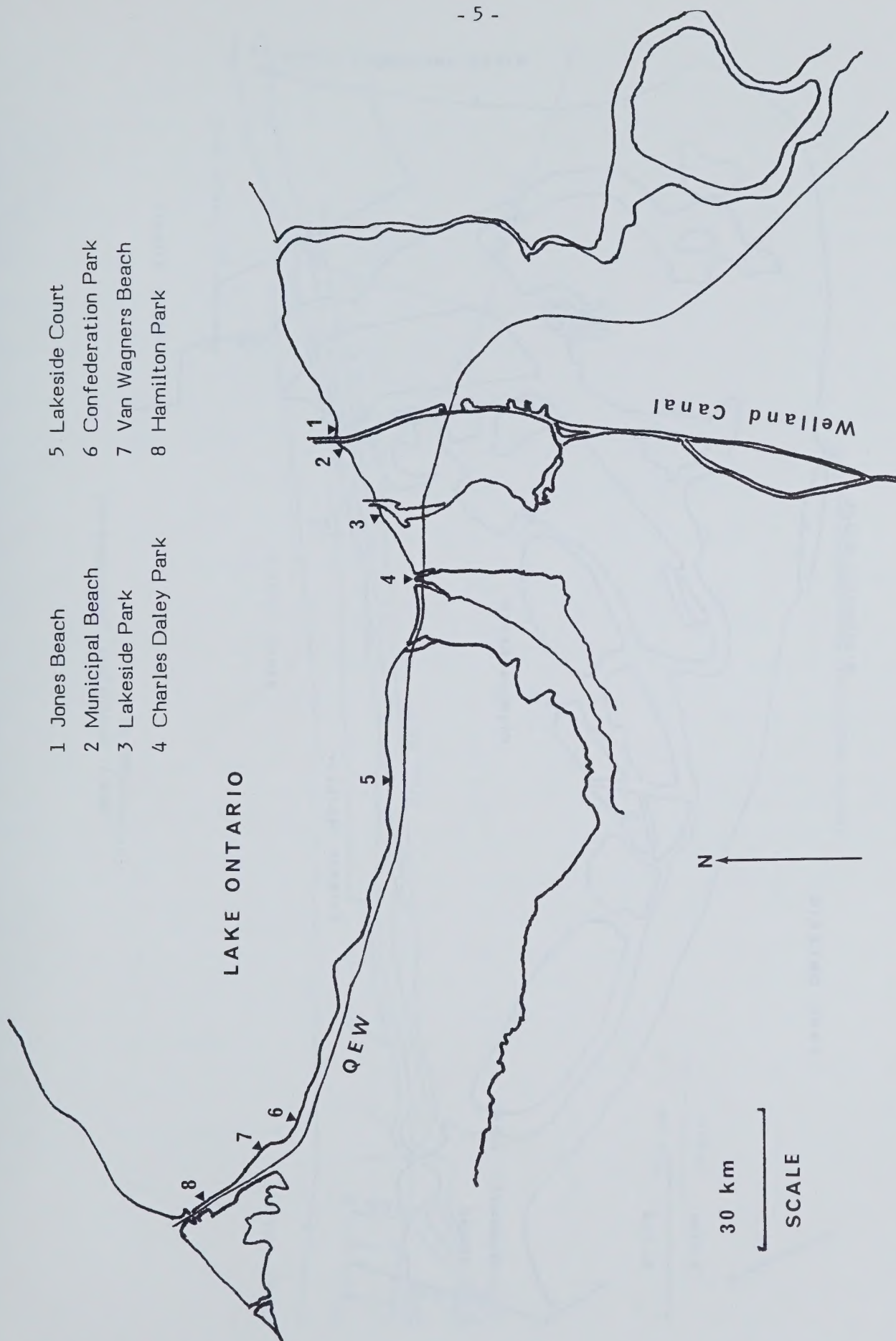


Figure 2 - Sampling Sites - Hamilton and St. Catharines Region

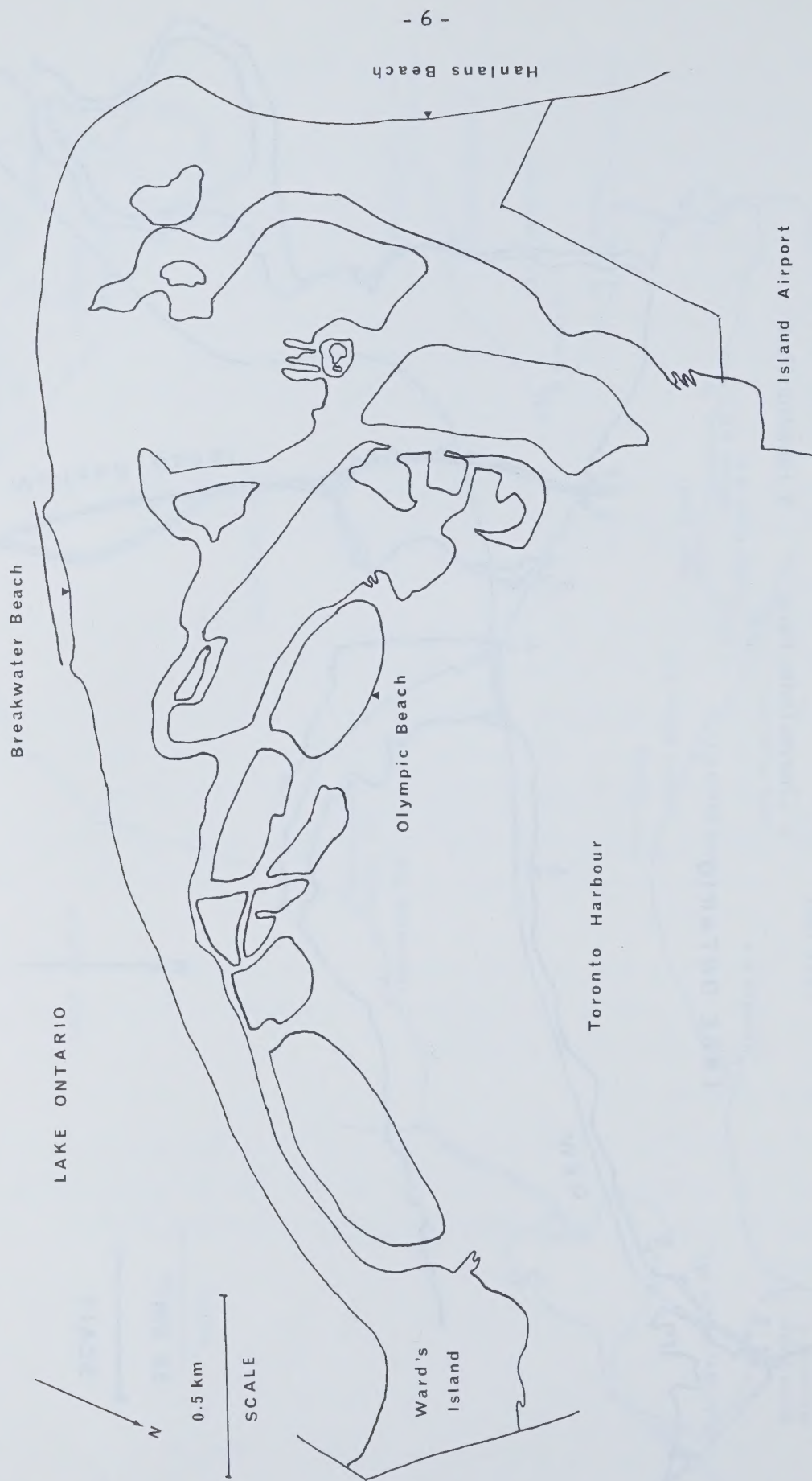


Figure 3 - Sampling Sites - Toronto Island Park

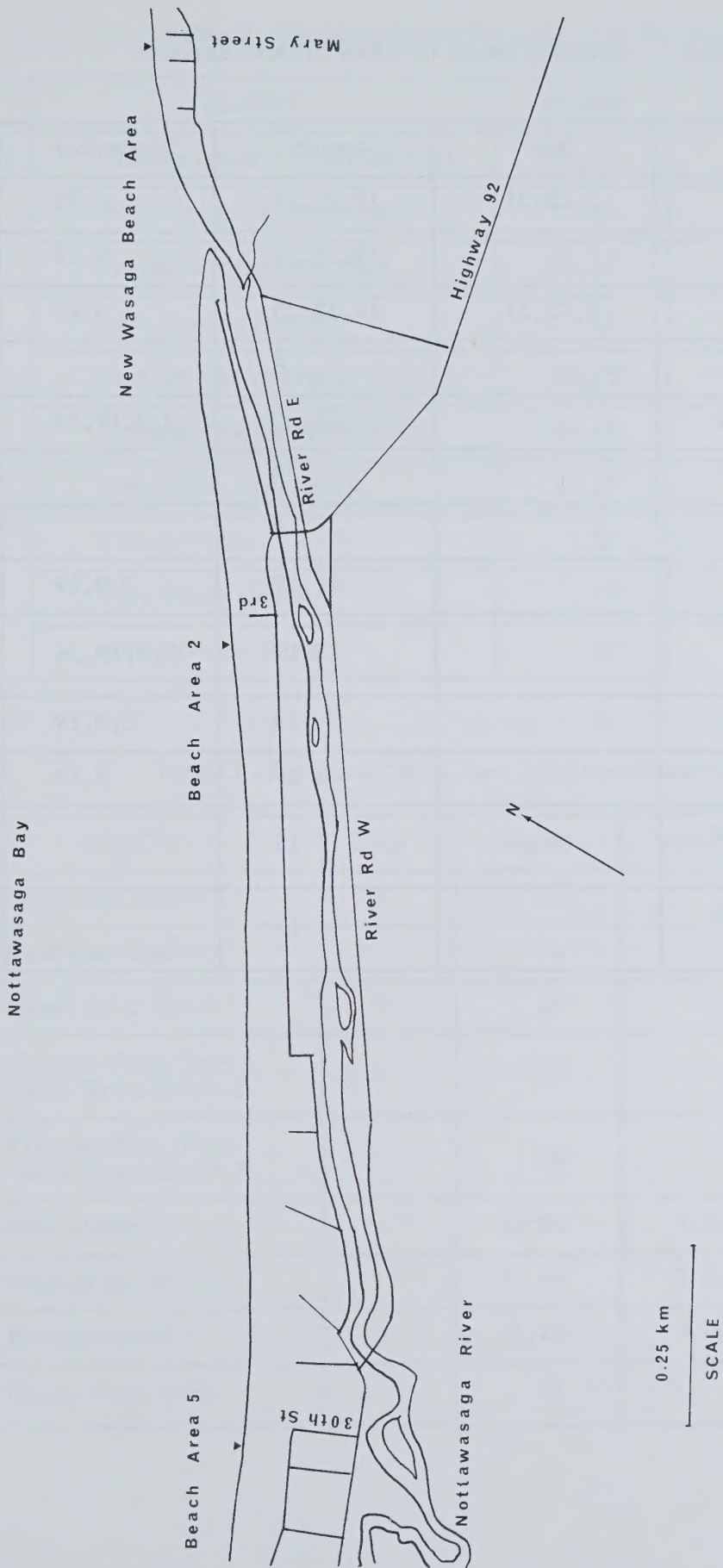


Figure 4 - Sampling Sites - Wasaga Beach Provincial Park

Table 1 - Sampling Dates for Lake Ontario Beaches

BEACH	July	August	September	October
Breakwater Beach	2,22,31	19,22,25	9,25	1
Hanlans Pt. Beach		19,22,25	9,25	1
Olympic Beach	2,22,31	19,22,25	9,25	
Jones Beach		8		10
Confederation Beach		8,12,19	2,9,19,26	
Hamilton Park		8		
Lakeside Park		8		10
Lakeside Court		12,19	2,9,19	
Municipal Beach (Weller Park)		12,19	2,9,19,26	
Charles Daley Park		19	2,9,19	
Van Wagner's Beach		19	9,19	
Darlington Prov. Park (Boat Dock)		15		
Darlington Prov. Park (Group Picnic Area)		15		

Table 2 - Sampling Dates for Lake Erie Beaches

BEACH	August	September
Holiday Beach Provincial Park	15,23	13
Point Pelee National Park	15,23	13
Rondeau Provincial Park	15,23	13
Port Stanley Beach	15	
Port Bruce Provincial Park	15,23	
Iroquois Provincial Park	15,23	3,13
Long Point Prov. Park	15,23	3,13
Turkey Point	23	3,13
Port Dover Beach	23	3,13
Selkirk Prov. Park	23	3

Table 3 - Sampling Dates for Lake Huron Beaches

BEACH	July	August	September	October
Ipperwash Beach I	7	21		21
Ipperwash Beach II		21		
Grand Bend Beach	7	21		21
Killbear Prov. Park Parry Sound Beach I		10		
Killbear Prov. Park Parry Sound Beach II		10		
New Wasaga		11,26	3,8,17	
Wasaga Beach 1-2		11,26	3,8,17	16
Wasaga Beach 5		11,26	3,8,17	16
Pinery Prov. Park		21		

The surface water samples were diluted as required in phosphate buffered water. Sediment samples were prepared for analysis using the method described by Gerba and Smith (1979, see Appendix). An improved sediment preparation method, developed in our laboratory and described in the Appendix, will be used for future studies.

Isolation and Enumeration Procedures

All methods are described in detail in the Appendix.

Fecal Coliforms

Each water sample was analyzed for the presence of fecal coliforms by the most probable number technique and the membrane filtration procedure. However, the m FC plates were inadvertently incubated at 35°C instead of 44.5°C and therefore the results are included merely for comparison and not as bona fide data. Another membrane filtration technique using m-TEC medium was used for the October 16 samples.

Sediment samples were evaluated using the most probable number technique.

Fecal Streptococci

Analysis of surface water samples was done using the membrane filtration technique with m Enterococcus agar and KF Streptococcus agar. The most probable number technique was employed for enumeration of fecal streptococci in sediment samples.

Heterotrophic Plate Count

Surface water and sediment samples were diluted appropriately in sterile phosphate buffered water and plated onto Heart Infusion (HI) agar (Difco) and Casein-Peptone-Starch (CPS) agar. Following incubation, the plates were counted and colonies were picked and transferred to Trypticase Soy agar (BBL) for further purification and classification according to the scheme described in the Appendix. Only the heterotrophic plate counts on CPS agar are reported here. The remainder of the data will be included in a separate report to follow.

Pseudomonas aeruginosa

The most probable number technique using asparagine broth was employed to analyze both surface water and sediment samples for P. aeruginosa. The enumeration method described by Seyfried and Fraser⁽¹⁷⁾ was also employed using the Ontario Ministry of the Environment's modified Drake's medium. A membrane filtration method using mPA, mPA-B, and mPA-C was included as well for selected samples.

Staphylococci and Coagulase Positive Staphylococcus aureus

The membrane filtration procedure using Vogel-Johnson agar with 0.5% sodium pyruvate added was used for the isolation of staphylococci from surface water. The presence or absence of coagulase positive Staphylococcus aureus in water and sediment was determined by pre-enriching the water and sediment samples in m Staphylococcus broth (Difco). Ten mL aliquots of water and weighed amounts of sediment were inoculated into Staphylococcus broth and incubated at 37°C for 24 hours. Appropriate dilutions of the broth culture were then spread onto Vogel-Johnson plates and incubated for 24 hours at 37°C. Typical staphylococcal colonies were then picked for further testing. The coagulase test was done initially using Difco Coagulase Plasma (Rabbit) and the reactions were confirmed using Coagulase Plasma EDTA (Rabbit). The staphylococcal isolates were also subjected to the following additional tests: Gram staining reaction; reaction on Mannitol Salts agar (Difco); hemolysis on sheep blood agar; production of pigment on TSA (BBL); DNase test on DNase Test agar with methyl green (Difco); lipase production on Colbeck EY agar (Difco); and thermo-nuclease test. These staphylococci data will be the subject of a further report.

Potentially Pathogenic Amoebae

Naegleria and Acanthamoeba species were isolated as described in Appendix A.

Enteric Viruses

Isolation and identification of enteric viruses was carried out as described in Appendix B.

Replication

Single samples, or in a few instances, duplicate samples were collected at each sampling site. Because of the workload involved in the processing of the samples, duplicate or triplicate microbiological determinations were not made. The results obtained using different dilutions or different volumes of sample are expressed as arithmetic mean values.

EPIDEMIOLOGICAL SURVEY

Three different beach survey techniques were tried in an attempt to determine the best method for optimum data recovery.

Questionnaire Procedures:

1. Initial interview conducted at beach using Questionnaire number 1 with telephone follow-up using Questionnaire number 1A. The people were contacted by telephone seven to ten days following the interview at the beach.

2. Initial interview conducted at beach (Questionnaire number 1) with Questionnaire number 1B mailed to those interviewed in lieu of telephoning. Because of the distance involved and the difficulty in reaching some of the participants by telephone, it was decided to mail out survey forms with self-addressed, stamped envelopes, shortly after the initial interview at the beach had been completed.
3. Questionnaire number 2 handed out to people at Provincial Parks. The questionnaires plus self-addressed, stamped envelopes were given to people on the beach and handed out to people as they arrived at the gate of the park. The participants were instructed to fill out the questionnaires and mail them at their earliest convenience.

The beaches at which interviews were conducted and the interviewing dates are shown in Table 4.

IPPERWASH CADET CAMP STUDY

A separate epidemiological survey was carried out at Ipperwash Cadet Camp on Lake Huron. This study was done to assess the overall effect of bathing on a large group of young people who were swimming at the same beach every day during July and August.

The Cadet Camp, located at Ipperwash Beach on Lake Huron, is under the jurisdiction of the Training Command in Trenton. The camp is adjacent to Ipperwash Provincial Park and south of Pinery Provincial Park. An area of the camp, locally called "Tent City", is also located on the beach and houses some of the staff members and their families (Figure 5). Approximately 1700 young cadets, were at the camp during the period from July 8 to August 27, 1979.

Microbiological Analyses

Water samples were collected twice during the summer by Ipperwash Camp personnel and sent to the Ministry of the Environment laboratory in London, Ontario for total coliform and fecal coliform analyses. Surface water and sediment samples were also analyzed in our laboratory on three separate occasions for the seven aforementioned microbial indicators.

Disease Incidence Data

On August 26, 1979, when all cadets had left Camp, the medical records were obtained from the Infirmary and the incidence of various types of illness tabulated. Illnesses due to common colds were not included in the data.

Questionnaire Number One

BEACH STUDY

GROUP NUMBER _____ DATE _____ TIME _____

LAKE _____ Sat., Sun., Mon., Tues., Wed., Thurs., Fri.

BEACH _____

Hello, I'm _____ . University of Toronto is working on a study to help improve swimming conditions at beaches throughout the province. We want to find out about conditions here at _____ Beach and we need the help of people who come to the beach today. We would like to ask about the experiences that you and your group have had at the beach today. Are you almost ready to leave the beach now? If no, contact them later.

1. During the past week, between Monday and Friday, did you or anyone at the beach with you now go in the water anywhere - here or at some other beach or pool?

YES (ask A & B)
NO (go to Q. 2)

A. Who was that? B. Did _____ (person) _____ actually swim, or get (his/her) head or face wet?

Record first name of each on a separate line.

Yes No (if NO record full name on page 2)

We are only interested now in those who did not actually swim or get their heads or faces wet during the past week between Monday and Friday.

2. What is your name and the names of the people who have not been swimming?
(complete Questions 3-9 for person 1 before completing questions for person 2, etc.)

	P1	P2	P3	P4	P5	P6
First Name						
Last Name						
Relationship to respondent						
Sex						
3. Code age without asking. If child or not sure, ask						
0 under 5						
1 5-9						
2 10-14						
3 15-19						
4 20-39						
5 40-49						
6 60 or over						
4. Sunday interview only Did _____ (person) _____ go swimming anywhere yesterday?						
IF YES:						
Did they have: their head under?						
head not under?						
Where did they swim?						
5. Did _____ (person) _____ go into the water at all today?						
IF YES:						
A. Did _____ (person) _____ actually swim or get his/her head or face wet?						

IF NO:

- B. Any physical symptoms such as
- 1 sunburn
 - 2 respiratory
 - 3 skin rash
 - 4 gastrointestinal
 - 5 other illness

IF A CHILD:

- C. Does he/she usually go into the water?

6. Could you please tell me if _____ (person) _____ was in the water around 1 or before; around 3; around 5 or after?

7. How long was _____ (person) _____ in the water?
We are only interested in the time actually in the water, not the total time at the beach.

- 1 less than 10
- 2 10-14
- 3 15-29
- 4 30-59
- 5 60 or more

8. During the past week did _____ (person) _____ have any of the following symptoms:
(ask everyone each symptom group)

- 1 sunburn
- 2 skin rash
- 3 backache, headache, fever
- 4 sore throat, cough, runny nose
- 5 vomiting, nausea, diarrhea
- 6 wheezing, or asthma-like attack
- 7 other symptoms

9. We are interested in whether or not anyone who is at the beach with you today gets any eye, ear, nose, throat, skin, or stomach trouble in the near future. Could you please give your complete name, address and telephone number so we can get in touch with you next week? IF NO PHONE: Is there any phone number where you can be reached - at a neighbor's or friend's phone, or at work? IF RELUCTANT TO GIVE PHONE: Could we reach you at work or at some other phone?

PRINT FULL NAME AND ADDRESS _____ ADDRESS (Street and No.) _____
NAME _____
City, Prov., Code _____
Home Phone _____ Other Phone _____ What is the best time to call? _____
Other Person's Names at this address _____, _____, _____, _____, _____

- 9A. What is (PERSON'S) address and phone number?

IF (PERSON) IS UNDER 16, AND NO RELATED ADULT IS LISTED, GET NAME, ADDRESS AND PHONE NUMBER OF A RESPONSIBLE ADULT AND SPECIFY RELATIONSHIP NEXT TO NAME.

NAME	ADDRESS	PHONE NUMBER	BEST TIME TO CALL
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

We appreciate your help very much. Thank you

Interviewer's Name _____

Questionnaire Number 1A

GROUP NUMBER _____

LAKE _____

BEACH _____

Telephone Follow-up

PERSON NUMBERS _____, _____, _____; PHONE _____

PERSON NUMBERS _____, _____, _____; PHONE _____

Hello, is this (CONTACT PERSON). (May I speak to (CONTACT PERSON))? This is _____.

I'm working with the Toronto University Beach Study. About a week ago one of our interviewers spoke

to you at _____ Beach. Do you recall?

GROUP NUMBER _____

NAME:

First, have you, _____, or _____ gone swimming or in the water anywhere -- at _____ Beach or any other beach, pool or lake - since the weekend we talked to you at the Beach?

YES (GO TO SECTION A)
NO (GO SECTION B, p. 3)

SECTION A

IF YES

Who was that? CIRCLE EACH NAME GIVEN.
Probe: Anyone else? CIRCLE NAMES

FOR EACH NAME CIRCLED ASK:

1. Did (PERSON) actually swim
(a) with their head out of the water, or
(b) get his/her head wet?
YES GO TO Q.2
NO ASK Q.1 FOR
NEXT NAME
CIRCLED

IF YES TO Q.1 ABOVE:

2. What days did they do that?
1. Sun.
2. Mon. - Fri.
3. Sat. or later

3. Where did (PERSON) go into the water?

P1	P2	P3	P4	P5	P6
YES 1 NO 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2	YES 1 NO 2
1 2	1 2	1 2	1 2	1 2	1 2
1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3

SECTION B

Now I would like to ask some questions about the people who were with you at the beach on the day of the interview who didn't swim at another beach during the following week.

That is, _____, _____, and _____. READ THE NAMES YOU HAVE NOT CIRCLED.

1. Let's start with (PERSON). HAS (PERSON) had any of these symptoms since you were at the beach on _____, _____ date ? ASK EACH SYMPTOM.

IF "YES" TO ANY OF THE FOLLOWING SYMPTOMS ASK:

What day did that start?

NAME:

PROBE: FIX EXACT DATE. WRITE DATE IN BOX UNDER THE PERSON AND BY THE SYMPTOM

	P1	Onset	P2	Onset	P3	Onset	P4	Onset	P5	Onset	P6	Onset
A. GASTROINTESTINAL												
1. Stomach ache	Y N		Y N		Y N		Y N		Y N		Y N	
2. Diarrhea or loose bowels	Y N		Y N		Y N		Y N		Y N		Y N	
3. Nausea or feeling nauseous	Y N		Y N		Y N		Y N		Y N		Y N	
4. Throwing up or vomiting	Y N		Y N		Y N		Y N		Y N		Y N	
B. RESPIRATORY												
1. Sore throat	Y N		Y N		Y N		Y N		Y N		Y N	
2. Bad cough	Y N		Y N		Y N		Y N		Y N		Y N	
3. Chest cold	Y N		Y N		Y N		Y N		Y N		Y N	
C. OTHER SYMPTOMS												
1. Fever (temperature) over 100°	Y N		Y N		Y N		Y N		Y N		Y N	
2. Headache lasting more than a few hours	Y N		Y N		Y N		Y N		Y N		Y N	
3. Backache	Y N		Y N		Y N		Y N		Y N		Y N	

GROUP NUMBER _____

NAME: _____

	P1	Onset	P2	Onset	P3	Onset	P4	Onset	P5	Onset	P6	Onset
D. EYE, EAR, NOSE												
1. Runny or stuffed nose	Y N		Y N		Y N		Y N		Y N		Y N	
2. Earache or runny ears	Y N		Y N		Y N		Y N		Y N		Y N	
3. Red, itchy, or watery eyes for more than one day, or styes	Y N		Y N		Y N		Y N		Y N		Y N	
E. ALLERGENIC												
1. Skin rash, itching skin, or welts	Y N		Y N		Y N		Y N		Y N		Y N	
2. Sneezing, wheezing, tightness in the chest breathlessness for more than a few minutes	Y N		Y N		Y N		Y N		Y N		Y N	
F. SUNBURN which bothered (you/him/her)	Y N		Y N		Y N		Y N		Y N		Y N	
2. IF NO SYMPTOMS, GO TO SECTION B, Q.1A (PAGE 3) FOR NEXT PERSON												
IF ANY DATE IS GIVEN, ASK Q's 3, 4, and 5:												
3. Did (PERSON) stay home because of (SYMPTOMS)?	Y N		Y N		Y N		Y N		Y N		Y N	
4. Did (PERSON) stay in bed?	Y N		Y N		Y N		Y N		Y N		Y N	
5. Did (PERSON) consult anyone for medical help? If so, could we please have the doctor's name, address, and telephone number?	Y N		Y N		Y N		Y N		Y N		Y N	

GO BACK TO Q. 1A (PAGE 3) FOR EACH ELIGIBLE PERSON.

GROUP NUMBER _____

We appreciate your help very much. The information you gave will help us in our study to improve swimming conditions at public beaches.

DATE OF COMPLETION: _____

TIME OF COMPLETION: _____

REASON FOR NONCOMPLETION:

	P1	P2	P3	P4	P5	P6
NO ANSWER						
NOT HOME						
WRONG NO. PROVIDED						
REFUSED WHEN CALLED						
DISCONNECTED NOS.						
NO TELEPHONE						
MOVED						
UNLISTED NO.						
OUT OF STATE						
REFUSED INFO. AT BEACH						

INTERVIEWER _____

Questionnaire Number 1B

University of Toronto Beach Study

Dear _____,

We are asking for your assistance in the final stage of our beach study. Approximately a week ago I spoke to you at _____ Beach. We would like to know whether or not you or your family or friends have developed any symptoms of illness since that time. Could you please answer the four questions on the following form and mail it back in the enclosed stamped envelope. My telephone is 978-2752 or 978-3732.

INTERVIEWER

First, have you _____, _____, _____, _____, _____ gone swimming at _____ Beach or at any other beach, pool or lake since we talked to you?

If YES - go to SECTION A

If NO - go to SECTION B

SECTION A

Please circle the names of the people who went swimming

QUESTION

1. For each name circled, did that person:
Swim with their head out of the water?
Get his or her head wet?
2. What days did they go swimming?
3. Where did they go swimming?

SECTION B

QUESTION

1. Did anyone who was at the beach with you on the day of the interview experience any of the symptoms listed below:

If YES, please write the date the symptoms started. Also note on the back of the page if the person stayed home, stayed in bed, or got medical help because of the symptoms.

	Date	Date	Date	Date	Date
A. <u>Gastrointestinal</u>					
1. Stomach ache					
2. Diarrhea or loose bowels					
3. Nausea or feeling nauseous					
4. Throwing up or vomiting					
B. <u>Respiratory</u>					
1. Sore throat					
2. Bad cough					
3. Chest cold					
C. <u>Other Symptoms</u>					
1. Fever (temperature) over 100°					
2. Headache lasting more than a few hours					
3. Backache					
D. <u>Eye, Ear, Nose</u>					
1. Runny or stuffed nose					
2. Earache or runny ears					
3. Red, itchy, or watery eyes for more than one day, or styes					
E. <u>Allergenic</u>					
1. Skin rash, itching skin, or welts					
2. Sneezing, wheezing, tightness in the chest, breathlessness for more than a few minutes					
F. <u>Sunburn</u>					

Thank you for your cooperation

University of Toronto Beach Study

We are students at the University of Toronto under the supervision of Professor P.L. Seyfried. During the past six years we have been investigating water pollution, studying primarily the bacteriological quality of lakes, rivers, and swimming pools. It has been observed in recent years that there tends to be an increase in illnesses and infections in the summer during peak swimming periods and there is speculation that this is directly related to contact with infectious organisms in swimming waters. We are interested in collecting data on the correlation between bacteria in the lake and the health of those persons swimming in the water. To collect this type of information, we require the cooperation of the swimmers, and we sincerely hope that you will be willing to take part in our study.

We would like to know whether you or your family or friends develop any symptoms of illness after swimming in the lake this weekend. Could you please answer the questions on the following form and mail it to us in the stamped envelope, or call me collect at (416) 978-2752 or 978-3732. All the information will be kept in strictest confidence, and each person will be identified as a number. Only the identification numbers will be used in any publication of the data.

We truly appreciate your assistance.

INTERVIEWER

GROUP NUMBER _____ BEACH _____ LAKE _____

	NAME	NAME	NAME	NAME	NAME
--	------	------	------	------	------

1. In each blank space please write in full the name of person who went swimming. Answer the questions concerning that person in the spaces below.

2. Did the person listed:
(a) swim with their head out of the water?

(b) get his or her head wet while swimming?

3. What days did they go in swimming?

4. Did they swim at any place other than this beach on the weekend or during the following week?

5. May we have your Doctor's Name _____

Telephone Number _____

Address _____

Did anyone who was at the beach experience, during the following week, any of the symptoms listed below?

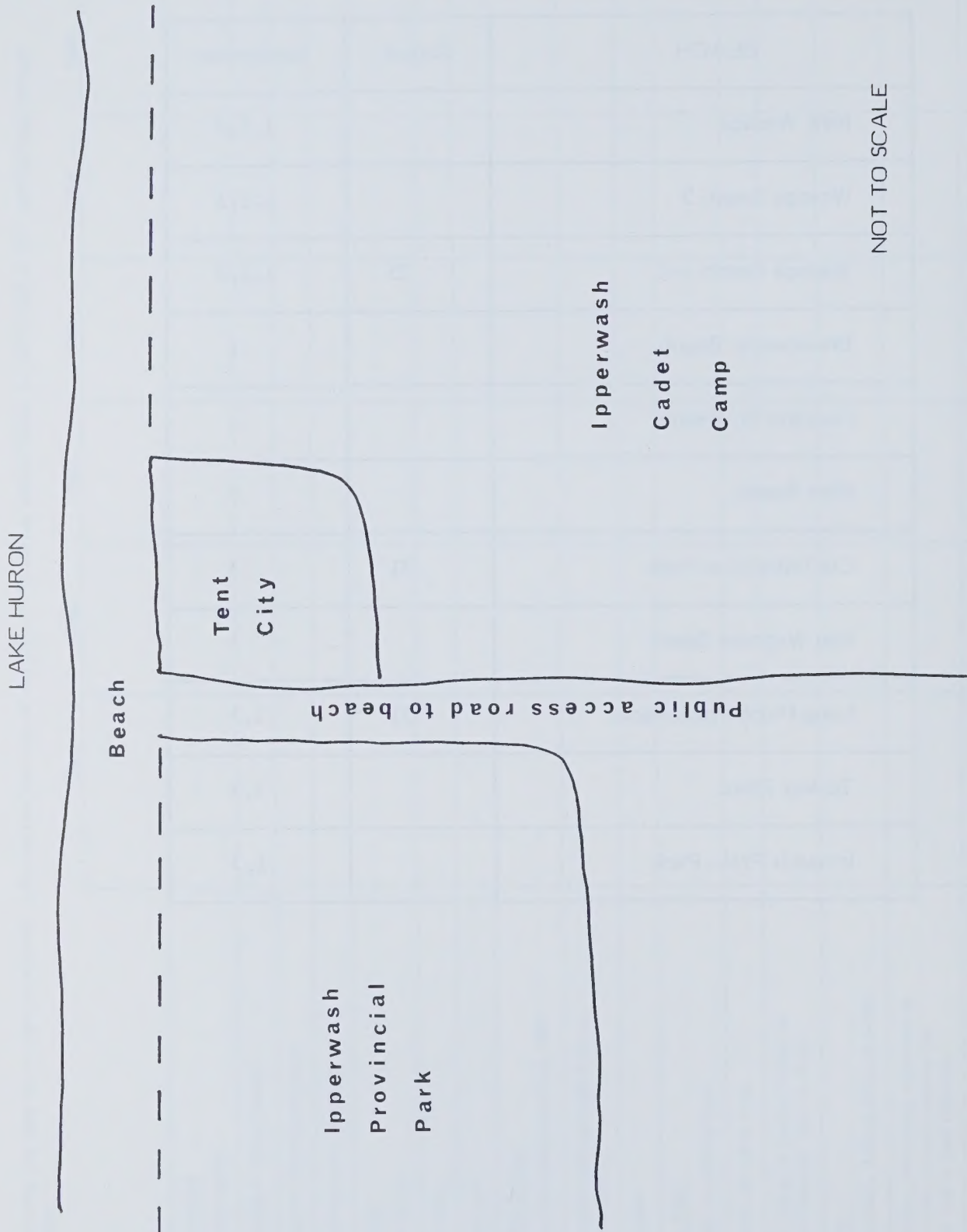
If YES, please write the date the symptoms started. Also note on the back of the page if the person stayed home, stayed in bed, or got medical help because of the symptoms.

	Date	Date	Date	Date	Date	Date
A. <u>Gastrointestinal</u>						
1. Stomach ache						
2. Diarrhea or loose bowels						
3. Nausea or feeling nauseous						
4. Throwing up or vomiting						
B. <u>Respiratory</u>						
1. Sore throat						
2. Bad cough						
3. Chest cold						
C. <u>Other Symptoms</u>						
1. Fever (temperature) over 100°						
2. Headache lasting more than a few hours						
3. Backache						
D. <u>Eye, Ear, Nose</u>						
1. Runny or stuffed nose						
2. Earache or runny ears						
3. Red, itchy, or watery eyes for more than one day, or styes						
E. <u>Allergenic</u>						
1. Skin rash, itching skin, or welts						
2. Sneezing, wheezing, tightness in the chest, breathlessness for more than a few minutes						
F. <u>Sunburn</u>						

Thank you for your cooperation

Table 4 - Interviewing Dates

BEACH	August	September
New Wasaga		1,2,3
Wasaga Beach 5		1,2,3
Wasaga Beach 1-2	25	1,2,3
Breakwater Beach		1
Hanlans Pt. Beach		1
Kew Beach		2
Confederation Park	31	3
Van Wagners Beach		3
Long Point Prov. Park	31	1,3
Turkey Point		1,3
Iroquois Prov. Park		1,3



NOT TO SCALE

Figure 5 - Relative Location of Cadet Camp and Provincial Park

RESULTS

BEACH LOCATIONS (ENVIRONMENTAL DATA)

The public beaches surveyed in the study ranged from locations with a high bather density, such as Grand Bend, to sites with relatively few swimmers, such as Port Dover. Likewise, high swimming loads were noted at Provincial Parks such as Wasaga in comparison with the few bathers found at Provincial Parks such as Darlington. Since the survey was begun late in the summer, some information on bather densities occurring during the height of the season was gathered from Park Superintendents and local residents.

A description of the number of bathers at each beach and the water and weather conditions at the time of sampling are noted in Tables 5, 6, and 7. The influence of air temperature on the number of bathers is illustrated in the description of bather load at Hanlans Point (Toronto Island) (Table 5). For example, on Sunday, July 22, when the air temperature was 28°C, there were 1002 people on the beach. At approximately the same time of day on a Saturday a month later, when the temperature had dropped to 20°C, there was no one on the beach. A similar comparison could be made at the Toronto Island Breakwater Beach, i.e., 1330 people at the beach on July 22 compared to 30 on August 25. At the Lake Erie beaches (Table 6), highest bather loads were recorded when the air temperature was in the 20° to 27°C range. Similarly, at Lake Huron beaches (Table 7), bather loads corresponded to air temperatures at or above 19°C.

As might be expected, the water temperatures at the Lake Erie beaches were the warmest, followed by Lake Huron and Lake Ontario. Water temperatures at beaches sampled on Lake Ontario during August 12 to September 19 ranged from 15°C to 21°C, with a mean temperature of 19°C. Beach sites on Lake Erie during August 15 to September 13, inclusive, had a range in water temperature of 18.5°C to 26°C and mean of 22°C. Lake Huron and Georgian Bay beaches during August 10 to September 17 had water temperatures ranging from 16° to 23°C, with a mean of 19.4°C. On the Labour Day weekend, comparative lake water temperatures at the beach sites sampled were: Lake Ontario, 18° to 21°C; Lake Erie, 22.5° to 26°C; and Lake Huron, 19° to 20°C.

PRELIMINARY STUDIES ON MICROBIAL ENUMERATION PROCEDURES

A number of different techniques for the isolation and enumeration of indicator organisms were evaluated during the course of the field study. Appropriate procedures for future work were established and are listed below. A brief summation of the reasons for selecting certain methods is also included.

Table 5 - Environmental Data for Lake Ontario Beaches, 1979

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			
								Number on Beach (not in water)	Number in Water		Total
									Head has not been immersed	Head has been immersed	
Hamilton	Hamilton Park	Thursday August 2	11:25 a.m.	21°C	17°C	Cloudy	Calm, murky	0	0	0	0
	Confederation Park	Thursday August 2	11:35 a.m.	21°C	17°C	Cloudy	Calm, some algal growth	0	0	0	0
		Sunday August 12	11:30 a.m.	19°C	17°C	Clear, sunny, windy	High waves, excessive algae	25	0	0	25
		Sunday August 12	3:30 p.m.	20°C	17.5°C	Clear, sunny, windy	High waves, less algae	193	1	1	195
		Sunday August 19	12:45 p.m.	20°C	17°C	Sunny, windy		220	0	0	220
		Sunday September 2	2:15 p.m.	22°C	18°C	Rain		0	0	0	0
	Sunday September 9	1:15 p.m.	19°C	19°C	Partial clouds		18	0	0	18	
	Wednesday September 19	12:25 p.m.	16°C	17.5°C	Windy	High waves, excessive algae	0	0	0	0	
	Wednesday September 26	12:00 noon	220°C	17°C	Sunny	Calm	0	0	0	0	
	Van Wagners Beach	Sunday August 19	1:15 p.m.	22°C	17°C	Sunny, windy		79	1	0	80
		Sunday September 9	1:30 p.m.	18°C	19°C	Sunny, partial clouds		3	0	0	3
		Wednesday September 19	12:45 p.m.	18°C	16°C	Windy, cloudy	Dirty, high waves	0	0	0	0

Table 5 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			Total
								Number on Beach (not in water)	Number in Water		
St. Catharines		Sunday August 12	2:00 p.m.	21°C	20°C	Sunny, clear, windy		49	0	4	53
		Sunday August 19	11:15 a.m.	21°C	20°C	Overcast	Excessive algae on beach, in water	5	0	0	5
		Sunday September 2	12:30 p.m.	22°C	21°C	Rain		0	0	0	0
		Sunday September 9	11:30 a.m.	18°C	18.5°C	Sunny, windy, partial clouds	Choppy	0	0	0	0
		Wednesday September 19	11:00 a.m.	16°C	16.5°C	Windy	High waves, cloudy water	0	0	0	0
	Lakeside Court	Wednesday September 26	10:45 a.m.	22°C	17°C	Sunny	Waves	0	0	0	0
		Sunday August 12	12:30 p.m.	18°C	18°C	Sunny, clear, windy	High waves	2	0	0	2
		Sunday August 19	12:30 p.m.	21°C	17°C	Sunny, windy	Calm, algae	0	6	0	6
		Sunday September 2	1:00 p.m.	22°C	21°C	Rain		6	0	0	6
		Sunday September 9	12:45 p.m.	17.5°C	18.5°C	Windy, cloudy	Excessive algae	0	0	0	0
	Jones Beach	Wednesday September 19	12:00 p.m.	13°C	15°C	Windy	High waves, some algae	0	0	0	0
		Thursday August 2	2:20 p.m.	23°C	22.5°C	Cloudy	Dense algal growth	0	0	3	3
		Wednesday October 10	12:10 p.m.	9°C	11.5°C	Windy, cloudy	Dirty, slimy excess algae	0	0	0	0

Table 5 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			Total
								Number on Beach (not in water)	Number in Water		
									Head has not been immersed	Head has been immersed	
St. Catharines (cont'd)	Lakeside Park	Thursday August 2	1:45 p.m.	22°C	22°C	Partly cloudy	Clear, calm	10	0	5	15
		Wednesday October 10	11:25 a.m.	9°C	11°C	Cloudy, windy	Clear, calm	0	0	0	0
	Charles Daley Park	Sunday August 19	12:00 noon	23°C	18°C	Sunny	Calm, clear	114	0	6	120
		Sunday September 2	1:45 p.m.	22°C	18°C	Rain		0	0	5	5
		Sunday September 9	12:00 noon	19°C	19°C	Cloudy	Choppy	2	0	0	2
		Wednesday September 19	11:30 a.m.	15°C	16.5°C	Windy	High waves, full of algae	0	0	0	0
Toronto Island	Breakwater Beach	Sunday July 22	11:50 a.m.	28°C	22°C	Sunny, some haze, no clouds	Clear, calm, some algae	628	32	229	889
		Sunday July 22	2:40 p.m.	27°C	22.5°C	Cloudy, strong breeze	Clear, calm some algae	956	114	260	1330
		Sunday July 22	5:15 p.m.	29°C	23.5°C	Sunny, slight breeze	Clear, calm, some algae	692	98	174	964
		Tuesday July 31	11:15 a.m.	23°C	20°C	Hot, hazy, humid, overcast	Clear	6	0	0	6
		Tuesday July 31	12:00 noon	24°C	20°C	Hot, hazy, humid, overcast	Clear	40	12	0	52
		Tuesday July 31	2:00 p.m.	26°C	20°C	Hot, hazy, humid, overcast	Clear	40	38	15	93
		Sunday August 19	1:00 p.m.	21°C	17°C	Sunny, hazy, humid	Clear, calm, odiferous, algae	121	16	0	137
		Wednesday August 12	2:30 p.m.	20°C	19°C	Sunny, clear breezy	Clear	226	1	27	254

Table 5 (cont'd)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts				Total
								Number on Beach (not in water)	Number in Water		Head has not been immersed	
									Head has been immersed	Head has not been immersed		
Toronto Island (cont'd)	Breakwater Beach (cont'd)	Saturday August 25	12:00 noon	22°C	18°C	Sunny, partial Cloud, windy	Clear	30	0	0	0	30
		Saturday September 8	12:30 p.m.	18°C	20°C	Sunny, clear, cold	Clear	6	0	0	0	6
		Tuesday September 25	1:00 p.m.	18°C	13°C	Sunny, slight breeze	Calm, clear	0	0	0	0	0
		Monday October 1	1:15 p.m.	17°C	14.5°C	Cloudy, hazy	Calm, clear	0	0	0	0	0
	Olympic Beach	Sunday July 22	1:05 p.m.	28°C	23.5°C	Sunny, some haze, no clouds	Clear, calm	138	6	23	23	167
		Sunday July 22	3:57 p.m.	27°C	24.5°C	Sunny, slight breeze	Clear, calm	81	23	84	84	188
		Sunday July 22	6:15 p.m.	28°C	24°C	Sunny, slight breeze	Clear, calm	66	14	30	30	110
		Tuesday July 31	11:00 a.m.	23°C	20°C	Hot, hazy, humid, overcast	Clear, calm	0	0	0	0	0
		Sunday August 19	1:45 p.m.	22°C	17°C	Sunny, hazy, humid	Clear, calm	10	0	0	0	10
		Wednesday August 22	3:00 p.m.	19°C	18.5°C	Sunny, clear, breezy	Clear	3	0	5	5	8
		Saturday August 25	12:30 p.m.	20°C	18°C	Sunny, partial cloud, windy	Clear	28	0	0	0	28
		Saturday September 8	1:00 p.m.	18°C	20°C	Sunny, clear, cold	Clear	0	0	0	0	0
		Tuesday September 25	1:30 p.m.	18°C	15°C	Sunny, clear, slight breeze	Clear, calm	0	0	0	0	0
		Sunday July 22	11:56 a.m.	28°C	23.5°C	Sunny, some haze, no clouds	Clear, calm	152	21	26	26	199
		Sunday July 22	3:26 p.m.	29°C	23.5°C	Clouds dispersing slight breeze	Clear, choppy	410	53	78	78	541

Table 5 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			
								Number on Beach (not in water)	Number in Water		Total
									Head has not been immersed	Head has been immersed	
Toronto Island (cont'd)	Ward's Island	Sunday July 22	5:49 p.m.	28°C	23.5°C	Sunny, slight breeze	Clear, calm	169	21	55	245
		Tuesday July 31	12:15 p.m.	24°C	20°C	Hot, hazy, humid, overcast	Clear, calm	41	4	0	45
		Tuesday July 31	2:15 p.m.	28°C	20°C	Hot, hazy, humid, overcast	Clear, calm	45	15	5	65
		Sunday August 19	1:00 p.m.	23°C	18°C	Sunny, hazy, humid	Clear, calm	23	0	0	23
	Hanlans Point	Sunday July 22	10:50 a.m.	26°C	23.5°C	Sunny, slight breeze	Clear, calm, some algae	188	12	30	230
		Sunday July 22	1:45 p.m.	28°C	23.5°C	Cloudy, strong breeze	Clear, choppy, some algae	858	89	55	1002
		Sunday July 22	4:25 p.m.	28.5°C	24°C	Sunny, slight breeze	Clear, calm, some algae	688	72	126	886
		Tuesday July 31	11:30 a.m.	24°C	20°C	Hot, hazy, humid, overcast	Clear, calm	8	5	7	20
		Tuesday July 31	1:00 p.m.	25°C	20°C	Hot, hazy, humid, overcast	Clear, calm	70	21	0	91
		Sunday August 9	1:00 p.m.	21°C	15.5°C	Sunny, slight breeze	Clear, calm	108	26	0	134
		Wednesday August 22	3:00 p.m.	23°C	19°C	Sunny, clear, breezy	Clear	156	0	0	156
		Saturday August 25	12:20 p.m.	20°C	20°C	Sunny, windy, partial cloud	Clear	0	0	0	0
		Saturday September 8	1:00 p.m.	20°C	19°C	Sunny, clear, cold	Clear	2	0	0	2
		Tuesday September 25	1:00 p.m.	19°C	13°C	Sunny, clear, slight breeze	Clear, calm	0	0	0	0

Table 5 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			Total
								Number on Beach (not in water)	Number in Water		
Oshawana		Monday October 1	12:00 noon	18°C	15.5°C	Cloudy, hazy	Clear, calm	0	0	0	0
	No. 1 Darlington Provincial Park No. 2	Wednesday August 15	12:15 p.m.	16°C	17°C	Cloudy	Calm, algae	0	0	0	0
		Wednesday August 15	12:45 p.m.	16°C	17°C	Cloudy	Calm, murky	0	0	0	0

Table 6 - Environmental Data for Lake Erie Beaches, 1979

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			Total
								Number on Beach (not in water)	Number in Water		
									Head has not been immersed	Head has been immersed	
		Wednesday August 15	12:30 p.m.	21°C	21°C	Sunny, partial cloud, windy	Some algae	18	0	0	18
	Holiday Beach	Thursday August 23	12:45 p.m.	24°C	22°C	Windy, cloudy	Dirty, high waves	10	1	0	11
		Thursday September 13	1:30 p.m.	26°C	22.5°C	Cloudy, hazy	Choppy, dirty, full of algae	0	0	0	0
	Point Pelee	Wednesday August 15	1:40 p.m.	22°C	20°C	Sunny	Clear	25	0	0	25
		Thursday August 23	1:45 p.m.	21°C	23°C	Cloudy, hazy		50	0	50	100
		Thursday September 13	2:30 p.m.	25°C	24°C	Sunny, hazy	Clear, choppy	0	0	0	0
	Rondeau Park	Wednesday August 15	3:15 p.m.	20°C	19°C	Sunny, partial clouds	Calm	50	0	0	50
		Thursday August 23	3:15 p.m.	23°C	23°C	Cloudy, hazy		95	0	47	142
		Thursday September 13	5:00 p.m.	22°C	22°C	Cloudy	High waves, numerous dead fish	0	0	0	0
	Port Stanley	Wednesday August 15	5:00 p.m.	20°C	21°C	Sunny	Shallow	15	2	0	17
	Port Bruce	Wednesday August 15	5:20 p.m.	20°C	18.5°C			40	0	3	43
		Thursday August 23	4:50 p.m.	20°C	21°C	Cloudy, hazy, windy	High waves	0	0	0	0
	Iroquois	Wednesday August 15	5:45 p.m.	19°C	19°C	Cloudy	Clear	0	0	0	0
		Thursday August 23	5:10 p.m.	20°C	22°C	Cloudy, hazy, windy	High waves	20	0	18	38

Table 6 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts			Total
								Number on Beach (not in water)	Number in Water	Head has not been immersed	Head has been immersed
	Iroquois	Monday September 3	12:30 p.m.	26°C	25°C	Sunny, hazy, had rained previously	High waves, dirty	48	0	32	80
		Thursday September 13	6:30 p.m.	20°C	20°C	Cloudy	Choppy	0	0	0	0
	Long Point	Wednesday August 15	6:30 p.m.	18°C	19°C	Cloudy	Oily	0	0	0	0
		Thursday August 23	6:00 p.m.	22°C	23.5°C	Cloudy, hazy, windy	Shallow	0	0	0	0
	Turkey Point	Monday September 3	2:30 p.m.	27°C	26°C	Sunny, hazy, had rained earlier	High waves, clean, clear	45	0	23	68
		Thursday September 13	7:20 p.m.	19°C	21.5°C	Cloudy	Choppy	0	0	0	0
		Thursday August 23	6:25 p.m.	19°C	22.5°C	Windy, cloudy, had rained		4	0	0	4
		Monday September 3	4:15 p.m.	24°C	23.5°C	Sunny, hazy, had rained	High waves, dirty	200	0	31	231
	Port Dover	Thursday September 13	7:40 p.m.	18°C	22.5°C	Cloudy	Dirty, with grass & algae	0	0	0	0
		Thursday August 23	6:45 p.m.	19°C	22.5°C	Cloudy, light winds		10	0	6	16
		Monday September 3	5:30 p.m.	24°C	25°C	Sunny, hazy, earlier rain	High waves, dirty	45	0	10	55
		Thursday September 13	8:00 p.m.	19°C	22°C	Cloudy		0	0	0	0
	Selkirk	Thursday August 23	7:30 p.m.	18°C	22°C	Very windy	High waves	0	0	0	0
		Monday September 3	6:30 p.m.	23°C	22.5°C	Sunny, hazy, rained earlier	Dirty	0	0	0	0

Table 7 - Environmental Data for Lake Huron Beaches, 1979

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts				Total	
								Number on Beach (not in water)	Number in Water		Head has not been immersed		Head has been immersed
									Head has not been immersed	Head has been immersed			
Georgian Bay	New Wasaga	Saturday August 11	1:30 p.m.	19°C	21°C	Sunny, windy	Clear, choppy	46	4	18	68		
		Sunday August 26	2:00 p.m.	19°C	23°C	Sunny, partial cloud, slight breeze	Clear, calm	74	4	32	110		
		Monday September 3	3:30 p.m.	17°C	20°C	Cloudy, windy	Choppy	10	0	0	10		
		Saturday September 8	11:40 a.m.	16°C	17.5°C	Sunny, partial cloud, breeze	Clear, slightly choppy	0	0	0	0		
		Monday September 17	11:00 a.m.	20°C	17.5°C	Sunny, clear	High waves, dirty	0	0	0	0		
		Tuesday October 16	2:00 p.m.	10°C	11°C	Sunny, clear	Clear, calm	0	0	0	0		
	Wasaga Beach 1 and 2	Saturday August 11	2:00 p.m.	19°C	21.5°C	Sunny, windy	Clear, choppy	55	6	15	76		
		Sunday August 26	2:20 p.m.	20°C	23°C	Sunny, partial cloud	Clear, shallow	362	68	120	550		
		Monday September 3	3:25 p.m.	18°C	19°C	Cloudy, windy	Choppy	6	1	0	7		
		Saturday September 8	11:20 a.m.	16°C	18°C	Sunny, a few clouds, breezy	Clear, a little choppy	2	0	0	2		
Wasaga Beach 5	Monday September 17	10:30 a.m.	20°C	17.5°C	Sunny, clear	High waves, dirty	0	0	0	0			
	Tuesday October 16	1:30 p.m.	10°C	12°C	Sunny, clear	Clear, calm	0	0	0	0			
	Saturday August 11	3:15 p.m.	19.5°C	21°C	Sunny, windy	Clear, choppy	55	6	15	76			
	Sunday August 26	2:00 p.m.	19°C	23°C	Sunny, partial cloud, light breeze	Dirty at shore, clear 15 feet from shore	74	4	32	110			

Table 7 (cont'd.)

Area	Beach	Date	Time of Sampling	Air Temperature	Water Temperature	Weather Description	Water Description	Headcounts				Total
								Number on Beach (not in water)	Head has not been immersed	Head has been immersed	Number in Water	
Grand Bend	Wasaga Beach 5 (cont'd)	Monday September 3	3:35 p.m.	18°C	19°C	Cloudy, windy	Choppy, turbid	6	1	0	0	7
		Saturday September 8	11:00 a.m.	16°C	16°C	Sunny, a few clouds, breezy	Clear, a little choppy	4	0	0	0	4
		Monday September 17	10:00 a.m.	20°C	17.5°C	Clear, sunny	High waves, dirty	0	0	0	0	0
		Tuesday October 16	1:00 p.m.	8.5°C	10°C	Clear, sunny	Clear, calm	0	0	0	0	0
	Killbear Point	Friday August 10	7:00 p.m.	17°C	21°C	Sunny	Calm	0	0	0	4	4
		Saturday July 7	1:00 p.m.			Sunny	Calm					
	Grand Bend	Tuesday August 21	9:00 a.m.	22°C	18°C	Sunny	Calm	0	0	0	0	0
		Sunday October 21	11:45 a.m.			Partly cloudy, windy	Rough	0	0	0	0	0
	Ipperwash	Saturday July 7	12:20 p.m.			Sunny	Calm					
		Tuesday August 21	7:20 a.m.	15.5°C	18°C	Sunny	Calm	0	0	0	0	0
		Sunday October 21	9:50 a.m.			Sunny	Calm	0	0	1	1	1
	Pinery Park	Tuesday August 21	8:15 a.m.	19°C	18°C	Sunny	Calm	0	0	0	0	0

Fecal Coliforms

Water - Membrane filtration (MF) using m-TEC medium.

The m-TEC procedure for fecal coliform enumeration was reported by Pagel and Vlassoff, at the ASM 79th Annual Meeting, Los Angeles, 1979, to have the best overall performance in a comparison of three MF methods. One advantage of this method is the fact that recovery of stressed Escherichia coli cells is promoted by an initial resuscitation period of 2 hours at $35 \pm 0.5^{\circ}\text{C}$.

Sediment - Most Probable Number (MPN) using lactose broth and EC broth with incubation of the latter at 44.5°C .

Fecal Streptococci

Water - MF using KF Streptococcus agar

Sediment - MPN using Azide Dextrose broth with confirmation in Ethyl Violet Azide broth.

Heterotrophic Plate Count

Water and Sediment - CPS agar

Various heterotrophic enumeration methods were evaluated by Young and described in the ASTM Special Technical Publication 673 (Methodology for Biomass Determinations and Microbial Activities in Sediments, Litchfield and Seyfried, editors, pp 40-51). He found that CPS agar gave optimal recovery of heterotrophs from both water and sediment samples.

Pseudomonas aeruginosa

Water - MPN using modified Drake's medium with confirmation on Pseudosel agar and skim milk agar.

- MF using mPA, mPA-B and mPA-C will also be attempted, although difficulties were experienced previously with background counts in excess of 300 colonies.

Sediment - MPN using modified Drake's medium with confirmation on Pseudosel agar and skim milk agar.

Staphylococci and Coagulase Positive Staphylococcus aureus

Water - MF using Vogel-Johnson agar with 0.5% sodium pyruvate and 1% Actidione added. Coagulase testing carried out using Coagulase Plasma EDTA. This procedure, recommended by R. Alico's ASTM Task Group on Staphylococcus aureus, has been found to work well in our laboratory.

Sediment - A presence or absence procedure with pre-enrichment of the sediment in m-Staphylococcus broth followed by cultivation on Vogel-Johnson agar as above.

Potentially Pathogenic Amoebae

Water and Sediment - as described in Appendix A.

Enteric Viruses

Water and Sediment - as described in Appendix B.

MICROBIOLOGICAL ANALYSES

Microbial determinations obtained from the water and sediment at the beaches are recorded in Tables 8, 9 and 10. Results of all the enumerations are included in the tables to emphasize the day-to-day variation in counts that occurred. Fluctuations in surface water counts seemed particularly evident on days when strong onshore winds caused rough water and high waves. For example, Tables 5 and 8 show that comparatively higher surface water counts of all the bacterial parameters were obtained at Lake Ontario beaches on August 12 and September 19 when the lake was rough. Similarly, wave action at the Lake Huron and Georgian Bay beaches (Tables 7 and 10) on September 3 and September 17 appeared to cause a general increase in surface water counts of fecal coliforms and fecal streptococci, in comparison with the counts obtained on non-windy days. The data obtained from Lake Erie (Table 6) indicates that on the sampling dates August 23, September 3 and September 13, the lake was either choppy or very rough. There were relatively few calm days during the survey and therefore assessment of the effect of environmental factors on the surface water bacterial counts was not feasible.

The fecal coliform counts at Confederation Park (Table 8) typify the non-comparable bacterial recoveries that were observed, on occasion, from water and sediment samples. For example, on August 2, coliform counts were high in the sediment and low in the surface water samples. On August 12, this situation was reversed; high levels of fecal coliforms were isolated from the surface water, but counts in the sediment were lower by a factor of 10.

Enumeration of heterotrophic bacteria in surface water and sediment (Tables 8, 9 and 10) showed that the levels of heterotrophs were always highest in the sediment. Maximum heterotrophic plate counts were occasionally observed at the Lake Ontario beaches, but in general, the difference in counts between beaches on the three Great Lakes was negligible.

Pseudomonas aeruginosa organisms were isolated from the water and/or sediment sometime during the sampling period at all the beaches surveyed. The MPN of P. aeruginosa/100 mL or /100 gm was as high as 23 organisms on Lakes Ontario and Erie and 13 organisms on Lake Huron.

Table 8 - Microbiological Data for Lake Ontario Beaches, 1979

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCCI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI			POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water	MPN	MF ^(a)		/100 mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre-sence in Sedi-ment	COAGULASE + STAPH. AUREUS	
Hamilton Park	Thursday August 2		21	-		18	-	5	<2	108	-	-	-
	Thursday August 2		22	-		20	-	<2	<2	2	-	-	-
	Sunday August 12		1600	TNTC		TNTC	-	23	23	TNTC	-	-	-
	Sunday August 19		2	85		23	$1.0^3 \times 10^3$	$4.29^5 \times 10^5$	<2	8	+	ND	ND
Confederation Park	Sunday September 2		8	20		16	$4.09^3 \times 10^3$	$1.0^5 \times 10^5$	<2	28	+	ND	-
	Sunday September 9		2	12		2	$1.85^3 \times 10^3$	$4.85^5 \times 10^5$	<2	6	+	ND	ND
	Wednesday September 19		170	203		39	$1.05^4 \times 10^4$	$2.17^4 \times 10^4$	5	46	+	ND	-
	Wednesday September 26		2	16		0	$1.48^3 \times 10^3$	$8.0^6 \times 10^6$	8	48	+	-	-
Van Wagners Beach	Sunday August 19		<2	80		14	$8.0^2 \times 10^2$	$2.45^5 \times 10^5$	23	96	+	-	ND
	Sunday September 9		8	48		10	$2.22^3 \times 10^3$	$2.52^5 \times 10^5$	13	14	+	ND	ND
	Wednesday September 19		5	52		12	$5.5^3 \times 10^3$	$6.33^5 \times 10^5$	8	42	+	ND	-
Municipal Beach	Sunday August 12		2	100		8	-	<2	2	50	-	-	-
	Sunday August 19		2	150		52	$7.0^2 \times 10^2$	$1.33^5 \times 10^5$	2	-	+	-	ND
	Sunday September 2		11	13		4	$2.58^4 \times 10^4$	<2	2	76	+	ND	-

Table 8 (cont'd.)

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCOCCI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI			POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water		/100 gm Sedi-ment		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre-sence in Sedi-ment	COAGULASE + STAPH. AUREUS	
		MPN	MF(a)							Water	Sedi-ment		
	Sunday September 9	7	66	170	16	$2.01^3 \times 10^3$	$3.6^3 \times 10^3$	2	< 2	34	+	ND	+
	Wednesday September 19	240	670	240	85	$3.2^4 \times 10^5$	$1.89^5 \times 10^5$	5	13	30	+	ND	-
	Wednesday September 26	33	44	350	3	$8.4^4 \times 10^4$	$3.6^6 \times 10^6$	< 2	8	18	+	ND	-
	Sunday August 12	540	TNTC	40	TNTC	-	-	23	23	TNTC	-	-	-
Lakeside Court	Sunday August 19	< 2	80	280	9	$1.0^2 \times 10^2$	$8.7^5 \times 10^5$	< 2	8	260	+	-	ND
	Sunday September 2	23	13	49	2	$2.4^3 \times 10^3$	$5.3^5 \times 10^5$	2	2	76	+	1	6
	Sunday September 9	8	48	130	12	$1.52^3 \times 10^3$	$2.81^5 \times 10^5$	2	13	10	+	ND	+
	Wednesday September 19	110	212	240	48	$1.28^4 \times 10^4$	$4.6^5 \times 10^5$	5	5	122	+	-	2
Jones Beach	Thursday August 2	920	TNTC	62	150	-	-	23	8	30	-	-	-
	Wednesday October 10	110	203	>2400	26	$2.71^3 \times 10^3$	$1.35^5 \times 10^5$	5	13	31	+	ND	-
Lakeside Park	Thursday August 2	22	-	>2400	50	-	-	2	13	74	-	-	-
	Wednesday October 10	1600	560	130	31	$1.23^4 \times 10^4$	$1.1^4 \times 10^4$	2	5	102	-	2	-
	Sunday August 19	5	270	9	94	$2.0^2 \times 10^2$	$1.52^5 \times 10^5$	2	13	-	+	-	ND
	Sunday September 2	< 2	20	22	10	$2.8^3 \times 10^3$	$1.94^5 \times 10^5$	< 2	< 2	114	+	-	5

Table 8 (cont'd.)

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCOCI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI			POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water	MPN	MF ^(a)		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre- sence in Sedi- ment	COAGULASE + STAPH. AUREUS	
								Water	Sedi- ment				
Charles Daley Park	Sunday September 9	33	120	350	0/50 mL	$2.25^4 \times 10^5$	$1.52^5 \times 10^5$	<2	5	14	+	ND	ND
	Wednesday September 19	79	188	33	127	$6.0^3 \times 10^3$	$2.89^5 \times 10^5$	13	8	246	+	ND	-
	Monday July 2	-	TNTC	1600	15	-	-	-	-	60	-	-	-
Breakwater Beach	Sunday July 22	-	TNTC	>2400	64	-	-	23	23	31	-	-	-
	Tuesday July 31	1600	TNTC	>2400	70	-	-	<2	8	-	-	-	-
	Sunday August 19	<2	70	<2	37	$6.0^3 \times 10^3$	$1.96^5 \times 10^5$	<2	2	6	+	-	ND
	Wednesday August 22	240	470	>2400	397	$1.45^2 \times 10^2$	$4.24^6 \times 10^6$	<2	<2	108	+	-	ND
	Saturday August 25	350	-	>2400	56	$9.15^3 \times 10^3$	-	2	2	137	+	-	ND
	Saturday September 8	540	128	>2400	62	-	$2.44^5 \times 10^5$	8	2	20	+	-	-
	Tuesday September 25	23	25	>2400	9	$6.04^4 \times 10^4$	$1.51^6 \times 10^6$	<2	5	216	+	ND	-
	Monday October 1	130	166	>2400	200	$3.91^2 \times 10^2$	$2.07^6 \times 10^6$	<2	13	15	+	-	-
	Monday July 2	-	TNTC	1600	53	-	-	-	-	70	-	-	-
	Sunday July 22	2400	TNTC	>2400	13	-	-	<2	8	90	-	-	-
	Tuesday July 31	350	229	1600	6	-	-	23	23	-	-	-	-

Table 8 (cont'd.)

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCOCI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI				POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water		/100 gm Sedi-ment		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre-sence in Sedi-ment	COAGULASE + STAPH. AUREUS	Sedi-ment	
Olympic Beach	Sunday August 19	MPN	MF ^(a)	11	29	-	2.02 ⁵ x 10 ⁵	< 2	2	11	+	-	-	ND
	Wednesday August 22	540	90	≥2400	64	3.4 ³ x 10 ³	5.2 ⁵ x 10 ⁵	< 2	5	26	-	-	-	ND
	Saturday August 25	350	125	≥2400	89	1.05 ⁴ x 10 ⁴	-	< 2	13	30	+	-	-	ND
	Saturday September 8	49	21	≥2400	11	-	2.74 ⁵ x 10 ⁵	< 2	13	20	+	ND	-	-
	Tuesday September 25	13	86	≥2400	3	2.19 ⁴ x 10 ⁴	3.07 ⁶ x 10 ⁶	2	5	4	+	ND	ND	-
Hanlans Point	Sunday August 19	< 2	170	13	15	1.05 ³ x 10 ³	3.4 ⁴ x 10 ⁴	13	2	102	+	-	-	-
	Wednesday August 22	< 2	-	540	-	9.5 ² x 10 ²	1.34 ⁵ x 10 ⁵	5	< 2	10	-	-	-	ND
	Saturday August 25	170	840	140	356	2.8 ⁴ x 10 ⁴	6.8 ⁵ x 10 ⁵	13	2	281	+	ND	-	+
	Saturday September 8	220	41	220	28	-	5.5 ⁴ x 10 ⁴	< 2	< 2	36	+	9	-	+
	Tuesday September 25	33	100	≥2400	15	4.3 ³ x 10 ³	4.6 ⁵ x 10 ⁵	2	5	4	+	ND	ND	-
Darlington Park No. 1	Monday October 1	5	24	540	1	2.4 ⁴ x 10 ⁴	2.8 ⁵ x 10 ⁵	2	13	1	+	-	-	-
	Wednesday August 15	350	320	240	39	1.39 ⁴ x 10 ⁴	1.33 ⁶ x 10 ⁶	23	23	70	-	-	-	ND
	Wednesday August 15	170	675	180	62	1.33 ⁴ x 10 ⁴	1.55 ⁴ x 10 ⁴	23	23	49	-	-	-	+

- Not Done + Present ND Not Detected
MPN Most Probable Number (a) Incubated at 35°C

Table 9 - Microbiological Data for Lake Erie Beaches, 1979

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCICI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI				POTENTIALLY PATHOGENIC AMOEBAE
		MPN	MF ^(a)	/100 mL Water		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre- sence in Sedi- ment	COAGULASE + STAPH. AUREUS	Sedi- ment	
Holiday Beach	Wednesday August 15	33	40	540	4	$2.85^3 \times 10^3$	$8.03^4 \times 10^4$	<2	8	-	-	-	-	ND
	Thursday August 23	8	60	5	55	$1.62^4 \times 10^4$	$4.95^5 \times 10^5$	2	8	223	-	-	-	ND
	Thursday September 13	49	100	27	49	$4.5^4 \times 10^4$	$2.70^5 \times 10^5$	5	2	308	+	ND	ND	-
Point Pelee	Wednesday August 15	13	130	49	4	$1.25^3 \times 10^3$	$2.69^4 \times 10^4$	8	13	360	+	-	-	ND
	Thursday August 23	27	140	130	14	$6.02^3 \times 10^3$	$2.18^5 \times 10^5$	5	5	115	-	ND	-	ND
	Thursday September 13	27	50	8	4	$2.25^3 \times 10^3$	$7.55^4 \times 10^4$	2	8	9	+	ND	-	-
Rondeau	Wednesday August 15	5	40	8	63	$1.6^2 \times 10^2$	$9.9^3 \times 10^3$	2	13	38	+	-	-	ND
	Thursday August 23	+2	50	33	38	$1.14^3 \times 10^3$	$2.9^5 \times 10^5$	<2	<2	80	-	-	-	+
	Thursday September 13	70	60	49	19	$3.18^3 \times 10^3$	$9.75^4 \times 10^4$	<2	<2	40	+	ND	-	-
Port Stanley	Wednesday August 15	170	25	40	10	$9.0^2 \times 10^2$	$2.06^4 \times 10^4$	5	5	28	-	-	-	ND
Port Bruce	Wednesday August 15	33	90	110	58	$4.0^2 \times 10^2$	$2.08^4 \times 10^4$	13	5	380	+	-	-	ND
Iroquois	Thursday August 23	13	50	22	13	$1.02^3 \times 10^3$	$1.33^5 \times 10^5$	<2	2	134	-	ND	-	ND
	Wednesday August 15	33	135	95	8	$1.15^3 \times 10^3$	$2.06^4 \times 10^4$	23	8	30	-	-	-	ND
	Thursday August 23	130	170	130	39	$1.01^3 \times 10^3$	$2.65^5 \times 10^5$	<2	2	123	-	ND	-	ND

Table 9 (cont'd.)

Beach	Date	FECAL COLIFORMS		FECAL STREP-TOCOCOCCI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI			POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water	/100 gm Sedi-ment		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre-sence in Sedi-ment	COAGULASE + STAPH. AUREUS	
		MPN	MF ^(a)						Water	Sedi-ment		
Iroquois	Monday September 3	17	55	20	2.96 ³ x 10 ³	2.18 ⁵ x 10 ⁵	5	2	2	72	2	17
	Thursday September 13	79	25	109	2.55 ² x 10 ²	1.03 ⁵ x 10 ⁵	2	<2	ND	612	ND	-
Long Point	Wednesday August 15	46	230	56	1.35 ³ x 10 ³	2.90 ³ x 10 ³	2	13	-	194	-	ND
	Thursday August 23	23	60	23	4.02 ³ x 10 ²	6.73 ⁴ x 10 ⁴	5	2	ND	152	ND	ND
	Monday September 3	1600	110	36	6.0 ³ x 10 ³	1.19 ⁵ x 10 ⁵	13	2	ND	88	ND	-
	Thursday September 13	130	-	29	6.42 ³ x 10 ²	9.95 ³ x 10 ³	1	5	ND	28	ND	-
Turkey Point	Thursday August 23	6	80	65	5.5 ³ x 10 ³	6.9 ⁵ x 10 ⁵	<2	2	-	106	-	+
	Monday September 3	49	50	2	2.17 ⁴ x 10 ⁴	2.5 ⁵ x 10 ⁵	8	2	ND	208	ND	-
	Thursday September 13	17	40	12	5.3 ³ x 10 ³	1.13 ⁵ x 10 ⁵	2	5	ND	52	ND	-
	Thursday August 23	540	265	278	1.2 ³ x 10 ³	2.02 ⁵ x 10 ⁵	<2	2	-	468	-	ND
Port Dover	Monday September 3	1600	TNTC	82	-	-	23	8	ND	412	ND	-
	Thursday September 13	920	890	76	1.18 ³ x 10 ³	6.16 ⁴ x 10 ⁴	8	2	ND	380	ND	-
Selkirk	Thursday August 23	39	340	382	6.2 ³ x 10 ³	3.39 ⁵ x 10 ⁵	8	<2	ND	TNTC	ND	ND
	Monday September 3	2400	290	72	1.72 ⁴ x 10 ⁴	1.29 ⁵ x 10 ⁵	23	13	ND	72	ND	-

- Not Done / + Present / ND Not Detected / MPN Most Probable Number / (a) Incubated at 35°C

Table 10 - Microbiological Data for Lake Huron Beaches, 1979

Beach	Date	FECAL COLIFORMS			FECAL STREP-TOCOCCHI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI				POTENTIALLY PATHOGENIC AMOEBAE
		/100 mL Water	/100 gm Sedi-ment	/100 mL Water		/mL Water	/gm Sedi-ment	/100 mL Water	/100 gm Sedi-ment	/100 mL Water	Pre- sence in Sedi- ment	COAGULASE + STAPH. AUREUS	Sedi- ment	
New Wasaga	Saturday August 11	MPN 31	MIF ^(a) 4	10	-	-	-	2	2	3	-	-	-	-
	Sunday August 26	110	60	18	5.66 ³ x 10 ³	4.89 ⁵ x 10 ⁵		2	2	181	+	-	-	ND
	Monday September 3	170	47	7	3.1 ³ x 10 ³	6.55 ⁴ x 10 ⁴		2	2	108	+	10	4	-
	Saturday September 8	23	12	10	1.14 ³ x 10 ³	3.25 ³ x 10 ³		2	2	4	+	-	-	ND
	Monday September 17	240	88	36	2.96 ³ x 10 ³	8.15 ⁴ x 10 ⁴		2	2	84	+	-	-	-
	Tuesday October 16	4	2	4	8.54 ³ x 10 ³	9.5 ⁴ x 10 ⁴		2	5	14	+	ND	ND	-
	Saturday August 11	49	39	14	-	-		2	2	20	-	-	-	-
Wasaga Beach 1 and 2	Sunday August 26	240	170	12	5.05 ³ x 10 ³	9.6 ⁵ x 10 ⁵		2	2	220	+	-	-	ND
	Monday September 3	180	250	38	4.6 ³ x 10 ³	4.2 ⁵ x 10 ⁵		13	2	100	+	ND	-	-
	Saturday September 8	33	16	0	1.29 ³ x 10 ³	1.57 ⁵ x 10 ⁵		2	2	20	+	-	-	+
	Monday September 17	47	120	35	3.55 ³ x 10 ³	1.36 ⁵ x 10 ⁵		2	2	40	+	-	-	-
	Tuesday October 16	2	2	0	1.24 ³ x 10 ³	1.43 ⁵ x 10 ⁵		13	5	2	+	ND	ND	-
	Saturday August 11	70	44	6	-	-		+2	+2	21	-	-	-	-
	Sunday August 26	130	290	31	1.04 ⁴ x 10 ⁴	3.78 ⁵ x 10 ⁵		+2	+2	41	+	-	-	ND

Table 10 (cont'd.)

Table 10 (cont'd.)														
Beach	Date	FECAL COLIFORMS			FECAL STREP- TOCOCOI	HETEROTROPHIC PLATE COUNT		PSEUDOMONAS AERUGINOSA		STAPHYLOCOCCI				POTEN- TIALY PATHO- GENIC AMOE- BAE
		/100 mL Water		/100 gm Sedi- ment		/100 mL Water	/gm Sedi- ment	/100 mL Water	/100 gm Sedi- ment	Pre- sence in Sedi- ment	COAGULASE + STAPH. AUREUS			
											Water	Sedi- ment		
Wasaga Beach 5	Monday September 3	MPN 540	MF(a) 250	52	2.63×10^3	1.765×10^5	2	2	108	+	-	-	-	
	Saturday September 8	33	29	28	2.83×10^3	1.435×10^5	< 2	2	56	+	-	-	+	
	Monday September 17	140	120	48	1.284×10^4	8.954×10^4	< 2	< 2	76	+	ND	-	-	
	Tuesday October 16	49	24	7	3.233×10^3	2.125×10^5	2	2	21	+	ND	1	-	
Killbear Point No. 2	Friday August 10	46	20	6	6.02×10^2	1.595×10^5	5	2	2	-	ND	-	-	
	Friday August 10	33	56	6	1.02×10^2	2.924×10^4	< 2	13	11	-	-	-	-	
Grand Bend	Saturday July 7	-	-	40	-	-	-	-	90	-	-	-	-	
	Tuesday August 21	130	70	110	2.03×10^3	-	2	2	10	-	-	-	ND	
	Sunday October 21	11	-	-	-	-	-	-	-	-	-	-	-	
Ipierwash	Saturday July 7	-	-	-	-	-	-	-	-	-	-	-	-	
	Tuesday August 21	540	280	253	5.173×10^3	1.784×10^4	13	2	248	+	-	-	ND	
	Sunday October 21	2	-	-	-	-	-	-	-	-	-	-	-	
Pinery Park	Tuesday August 21	38	50	12	-	-	< 2	2	57	-	-	-	ND	

- Not Done + Present ND Not Detected
MPN Most Probable Number (a) Incubated at 35°C

Staphylococci were also evident at each of the sampling locations. However, isolation of coagulase positive S. aureus was limited to Lakeside Court, Lakeside Park, Charles Daley Park and Hanlans Point on Lake Ontario; Iroquois Park and Long Point on Lake Erie; and New Wasaga and Wasaga 5 beaches on Lake Huron.

Amoebae capable of growth at 42°C were isolated from the following sources: Darlington Park, Hanlans Point, Municipal Park, and Lakeside Court on Lake Ontario; Rondeau Park, Turkey Point, Holiday and Mersea Beaches on Lake Erie; and Wasaga Beach on Lake Huron (Tables 8, 9 and 10). These potentially pathogenic organisms were submitted to further testing as described in the Naegleria Report (Appendix A).

The number of samples taken at the individual beaches and the geometric means of fecal coliforms, determined by two different methods in water, and the geometric means of the organisms in sediment are shown in Table 11. Ipperwash, Lake Huron; Port Dover, Lake Erie; and Lakeside Park and Jones Beach, Lake Ontario were found to have the highest levels of fecal coliforms present. The densities of fecal streptococci at these beaches were also high. Sample numbers and geometric means of fecal streptococci, staphylococci and Pseudomonas aeruginosa are depicted in Table 12.

A summation of bacterial indicator results is presented in Table 13. Here the beaches are listed in order of their relative pollution with respect to each of the indicator organisms. Of the Lake Erie beaches, Port Dover had consistently high counts of fecal coliforms in water and sediment as well as fecal streptococci, staphylococci and P. aeruginosa in the water. With the exception of fecal coliforms in the sediment at Turkey Point and fecal streptococci in the water at Rondeau Park, these two beaches could be described as being relatively unpolluted in terms of the parameters used.

From the microbial results, the St. Catharines beaches of Jones and Lakeside Park appeared to be more polluted than the Toronto Island beaches of Breakwater, Olympic and Hanlans Point. However, it should be noted that Municipal (Weller) Park, also in St. Catharines, and Confederation Park in Hamilton demonstrated the lowest levels of bacterial parameters.

Pollution levels at the Lake Huron beaches varied. Ipperwash Beach had the highest levels of all four indicators in the water. Grand Bend Beach was similar with the exception of relatively low P. aeruginosa counts in the water and sediment.

EPIDEMIOLOGICAL SURVEY

A total of 518 participants, including 479 swimmers and 39 nonswimmers, were surveyed at 10 beaches on three of the Great Lakes. The majority of the successful interviews were conducted on the Labour Day weekend. The illness rates for the swimmers who were interviewed

Table 11 - Geometric Means of fecal coliforms at the beaches sampled

BEACH	Organism	n	Geometric Mean	Organism	n	Geometric Mean	Organism	n	Geometric Mean
<u>Lake Huron Beaches</u>	FECAL COLIFORMS IN WATER/100 mL (M.P.N.)			FECAL COLIFORMS IN WATER/100 mL (M.F.)			FECAL COLIFORMS IN WATER/100 gm		
New Wasaga Beach		6	48.7*		6	17.0*		6	>1289
Wasaga Beach 1 and 2		6	43.3*		6	43.0*		6	>1071
Wasaga Beach 5		6	101.8*		6	80.2*		6	>1359
Killbear No. 1		1	46.0		1	20.0		1	>2400
Killbear No. 2		1	33.0		1	56.0		1	920
Grand Bend		2	37.8*	(130)	1	70.0		2	705* (920)
Ipperwash		2	32.9*	(540)	3	150.0**		3	587* (1600)
Pinery		1	38.0		1	50.0		-	-
<u>Lake Erie Beaches</u>									
Holiday		3	23.5		3	63.5		3	42
Point Pelee		3	21.5		3	96.9		3	37
Rondeau		3	8.9		3	49.3		3	23
Port Stanley		1	33.0		1	90.0		1	40
Port Bruce		2	20.7		2	67.1		2	49
Iroquois		4	49.0		4	74.9		4	319
Long Point		4	121.8		4	114.9		4	34
Turkey Point		3	17.1		3	56.6		3	77
Port Dover		3	926.0		2	>486.0		3	>908
Selkirk		2	306.0		2	31.40		2	253
<u>Lake Ontario Beaches</u>									
Hamilton Park		1	21.0		-	-		1	920
Confederation Park		7	16.8		6	>36.7		7	414
Van Wagners		3	4.3		3	64.5		3	105
St. Catharines		6	6.2		6	85.1		6	114
Municipal Beach		5	29.4		4	>57.0		5	111
Lakeside Court		2	318.0*	(920)	1	>203.0* (TNTC)		2	>386* (62)
Jones Beach		2	187.6*	(22)	1	560.0*		2	>559* (2400)
Lakeside Park		2	6.9		4	86.5		4	63.3
Charles Daley Park		4	116.7		6	>121.9		9	>1044
Breakwater		8	110.6		6	88.0		8	>1106
Olympic Beach		7	17.1		6	105.8		6	220
Hanlans Point		6	350.0		5	320.0		1	240
Darlington No. 1		1	170.0		1	675.0		1	180
Darlington No. 2		1							

* - One sample taken in October. The number in brackets is the mean without this sample.

** - Includes two samples done by the Ministry of the Environment.

n - Number of samples.

Table 12 - Geometric Means of fecal streptococci, staphylococci and *Pseudomonas aeruginosa* at the beaches sampled

BEACH	Organism	n	Geometric Mean	Organism	n	Geometric Mean	Organism	n	MEDIAN For: <i>Pseudomonas aeruginosa</i>	Organism	n	MEDIAN For: <i>Pseudomonas aeruginosa</i>
Lake Huron Beaches	FECAL STREPTOCOCCI / 100 mL WATER	6	7.37*	STAPHYLOCOCCI / 100 mL WATER	6	25.5*	PSEUDOMONAS AERUGINOSA / 100 mL WATER	6	2*	P. AERUGINOSA IN SEDIMENT Per 100 gm	6	2
		6	7.14*		6	29.8*		6	3*		6	2
		6	21.20*		6	45.0*		6	2*		6	2
		1	6.00		1	2.0		1	5		1	2
		1	6.00		1	11.0		1	<2		1	13
		2	66.30		2	30.0		1	2		1	2
		1	253.00		1	248.0		1	13		1	16
		1	12.00		1	57.0		1	<2		1	2
		3	22.10		2	262.0		3	2		3	8
		3	6.10		3	72.0		3	5		3	8
		3	35.70		3	49.5		3	<2		3	<2
		1	10.00		1	28.0		1	5		1	5
		2	27.50		2	226.0		2	7		2	3.5
Lake Ontario Beaches	FECAL STREPTOCOCCI / 100 mL WATER	4	28.50	STAPHYLOCOCCI / 100 mL WATER	4	113.0	PSEUDOMONAS AERUGINOSA / 100 mL WATER	4	3.5	P. AERUGINOSA IN SEDIMENT Per 100 gm	4	2
		4	34.00		4	92.3		4	3.5		4	2
		3	11.60		3	105.3		3	2		3	2
		3	120.10		3	418.0		3	8		3	2
		2	165.80		1	>72.0		2	15.5		2	7
		1	18.00		1	108.0		1	5		1	<2
		6	>9.10		6	>20.4		7	<2		7	8
		3	11.90		3	38.3		3	8		3	13
		6	13.70		5	34.4		6	2		6	2
		4	>10.10		4	<70.0		5	2		5	13
		2	62.4*		2	30.5*		2	14*		2	10.5
		2	39.4*		2	86.8*		2	2*		2	(8)
		4	18.60		4	73.2		4	(23)		4	(13)
Other Beaches	FECAL STREPTOCOCCI / 100 mL WATER	9	56.80	STAPHYLOCOCCI / 100 mL WATER	8	42.5	PSEUDOMONAS AERUGINOSA / 100 mL WATER	8	<2	P. AERUGINOSA IN SEDIMENT Per 100 gm	8	6.5
		8	19.70		7	23.8		7	<2		7	3.5
		5	18.60		6	18.6		6	<2		6	5
		1	39.00		1	70.0		1	3.5		1	3.5
		1	62.00		1	49.0		1	23		1	23
		1	62.00		1	49.0		1	23		1	23
		1	62.00		1	49.0		1	23		1	23
		1	62.00		1	49.0		1	23		1	23
		1	62.00		1	49.0		1	23		1	23
		1	62.00		1	49.0		1	23		1	23

* - One sample taken in October. The number in brackets is the mean without this sample.
n - Number of samples.

Table 13 - Order of Beach Pollution

Fecal Coliforms (M.P.N.)	Fecal Coliforms (M.F.)	Fecal Coliforms (Sediment)	Fecal Streptococci	Staphylococci [†]	Ps. aeruginosa In Water	Ps. aeruginosa In Sediment	Decreasing LAKE ERIE →
Port Dover Selkirk Long Point Port Stanley Point Pelee Iroquois Holiday Beach Point Bruce Turkey Point Rondeau	Port Dover Selkirk Long Point Port Stanley Point Pelee Iroquois Holiday Beach Point Bruce Turkey Point Rondeau	Port Dover Iroquois Selkirk Long Point Port Bruce Holiday Beach Port Stanley Point Pelee Long Point Rondeau	Selkirk Port Dover Rondeau Long Point Port Bruce Iroquois Holiday Beach Turkey Point Port Stanley Point Pelee	Port Dover Holiday Beach Iroquois Port Bruce Turkey Point Long Point Point Pelee Rondeau Port Stanley --	Selkirk Port Dover Port Bruce Port Stanley Point Pelee Iroquois Long Point Turkey Point Holiday Beach Rondeau	[Holiday Beach Pointe Pelee Selkirk Port Stanley Port Bruce Iroquois Long Point Turkey Point Port Dover Rondeau	Decreasing LAKE ERIE →
Jones ¹ Darlington No. 1 Jones ² Lakeside Park ² Darlington No. 2 Breakwater Olympic Lakeside Court ¹ Hanlans Point Confederation Charles Daley Park Municipal Park* Van Wagners --	Jones ¹ Darlington No. 2 Lakeside Park ³ Darlington No. 1 Jones ² Breakwater ⁺ Hanlans Point Olympic ⁺ Charles Daley Park Municipal Park* Van Wagners Lakeside Court ⁺ Confederation ⁺ -- --	Lakeside ¹ Breakwater Olympic Hamilton Park Lakeside Park ² Confederation Jones ² Darlington No. 1 Hanlans Point Charles Daley Park Darlington No. 2 Municipal Park* Van Wagners Lakeside Court Confederation ⁺ --	Jones ¹ Jones ² Darlington No. 2 Breakwater Lakeside Park ¹ Lakeside Park ² Darlington No. 1 Olympic Hanlans Point Charles Daley Park Hamilton Park Municipal Park* Van Wagners Lakeside Court ⁺ Confederation ⁺	Hamilton Park Lakeside Park ² Lakeside Park ¹ Charles Daley Park Darlington No. 1 Lakeside Court ⁺ Darlington No. 2 Breakwater Van Wagners Municipal Park* Jones ¹ Jones ² Olympic Confederation ⁺ Hanlans Point	[Darlington No. 1 Darlington No. 2 Van Wagners Lakeside Park ² Jones ² Lakeside Park ² Confederation Jones ² Charles Daley Park Olympic Breakwater Hanlans Point Municipal Park* Hamilton Park	[Darlington No. 1 Darlington No. 2 Van Wagners Lakeside Park ² Jones ² Lakeside Park ² Confederation Jones ² Charles Daley Park Olympic Breakwater Hanlans Point Municipal Park* Hamilton Park	Decreasing LAKE ONTARIO →
Ipperwash ¹ Grand Bend ¹ Wasaga Beach ^{5 2} Killbear No. 1 New Wasaga ² Wasaga Beach ^{1 & 2} Pinery Wasaga Beach ^{1 & 2} Killbear No. 1 Killbear No. 2 Grand Bend ² Ipperwash ²	Ipperwash ¹ Wasaga Beach ^{5 2} Grand Bend ¹ Killbear No. 2 Pinery Wasaga Beach ^{1 & 2} Killbear No. 1 Killbear No. 2 Grand Bend ¹ New Wasaga ² -- --	Killbear No. 1 Ipperwash ¹ Wasaga Beach ^{5 2} New Wasaga ² Wasaga Beach ^{1 & 2} Killbear No. 2 Killbear No. 1 Grand Bend ² Ipperwash ² (all above 500)	Ipperwash ¹ Grand Bend ¹ Wasaga Beach ^{5 2} Pinery New Wasaga ² Wasaga Beach ^{1 & 2} Killbear No. 1 Killbear No. 2 -- --	Ipperwash ¹ Grand Bend ¹ Wasaga Beach ^{5 2} Pinery New Wasaga ² Wasaga Beach ^{1 & 2} Killbear No. 2 Killbear No. 1 -- --	Ipperwash ¹ Killbear No. 1 Wasaga Beach ^{1 & 2} New Wasaga ² New Wasaga ^{5 2} Grand Bend ¹ Killbear No. 2 Pinery -- --	Ipperwash ¹ Killbear No. 2 Wasaga Beach ^{1 & 2} New Wasaga ² Wasaga Beach ^{5 2} Grand Bend ¹ Killbear No. 1 Pinery -- --	Decreasing LAKE HURON →

+ - Excluding at least one TNTC sample

1 - Excluding October samples, if taken

2 - Including October samples

3 - One sample, from October 10, only

† - Selkirk not included, because one of 2 samples was TNTC

⌊ - Indicates same number

* - Municipal Park (of City of St. Catharines)

and who responded to our questionnaires are given in Table 14. The dubious cases cited in Table 14 include those instances: (a) where it was questionable whether or not the illness was related to swimming; (b) where the person went swimming at another location following the interview; or (c) where there was a prolonged time lapse between the swimming event and the subsequent demonstration of symptoms.

Of the 479 respondents, 11 became ill enough to seek medical help and/or take time off school or work. (Four at Wasaga, one in Toronto, three in Hamilton, and three at Iroquois.)

The incidence of illness appeared to be highest (31.6%) at the Hamilton beaches. However, it should be recognized that only 38 swimmers were interviewed at this location. The illness rate of 21.7% shown at the Lake Erie beaches is probably more meaningful due to the fact that 226 swimmers were canvassed during that survey. The lowest illness rate of 10.3% was recorded at the Toronto beaches.

Table 15 provides a breakdown of the symptom rates among the swimmers who became ill. Because each swimmer could experience more than one type of illness, a total of 186 symptoms were recorded for the 101 swimmers listed in Table 14. Correspondingly, 89 swimmers had 169 symptoms, if the dubious cases are taken into account.

Sunburn and eye, ear, and nose ailments were the most common complaints among the swimmers, followed by respiratory and gastrointestinal types of illnesses.

As may be seen from Table 15, the incidence of illness among swimmers at the Wasaga beaches was fairly evenly distributed between gastrointestinal, respiratory, and eye, ear, and nose symptoms. At the Toronto beaches, eye, ear, and nose complaints were most prevalent. Upper respiratory tract as well as eye and ear illnesses predominated at the Hamilton beaches. Finally, excluding sunburn, gastrointestinal and eye, ear and nose symptoms constituted the major forms of illness at the Lake Erie beaches.

Illness rates for the nonswimmers who participated in the survey are cited in Table 16. Five of the 39 nonswimmers developed illnesses and Table 17 indicated the distribution of their symptoms. Similar to the results observed for swimmers, the incidence of illness among the nonswimmers was highest (33.3%) at the Hamilton beaches.

Although the Wasaga beach swimming participants experienced a variety of illnesses, the only symptoms recorded for the nonswimmers were of a gastrointestinal nature. Nonswimmers at the Toronto beaches had no complaints of illness; and the Hamilton area nonswimmers suffered principally from respiratory ailments. Similar to the swimming group at the Lake Erie beaches, the types of illnesses experienced by the Lake Erie nonswimmers were widely scattered. The illness rate for nonswimmers at all beaches was 12.8% compared to 18.6% for swimmers.

Table 14 - Illness Rates for Swimmers at the Ten Beaches

Beach	No. of People	No. with Symptoms	Percentage
<u>Toronto Beaches</u>			
Breakwater	21	3	14.3 %
Hanlans Point	6	0	0 %
Kew Beach	2	0	0 %
Totals	29	3	10.3 %
<u>Hamilton Beaches</u>			
Confederation and Van Wagners Beach	38	16 (12)	42.1 % (31.6%)
<u>Lake Erie Beaches</u>			
Long Point	137	35*	25.5 %
Turkey Point	22	5	22.7 %
Iroquois	67	11 (9)	16.4 % (13.4%)
Totals	226	51 (49)	22.6 % (21.7%)
<u>Wasaga Beach</u>			
Wasaga Beach 5	98	15	15.3 %
Wasaga Beach 1&2	61	12 (7)	19.7 % (11.5%)
New Wasaga	27	4 (3)	14.8 % (11.1%)
Totals	186	31 (25)	16.7 % (13.4%)
Grand Total	479	101 (89)	21.1 % (18.6%)

* - Twelve had sunburn only.

The figures in brackets are the numbers with symptoms with all dubious cases removed.

Table 15 - Symptom Rates for Swimmers at the Ten Beaches

BEACH	No. with Gastro-intestinal Symptoms	%	No. with Respiratory Symptoms	%	No. with Ear, Eye, Nose Symptoms	%	No. with Allergic Symptoms	%	No. with Sun-burn	%	No. with Other Symptoms	%
<u>Wasaga Beach</u>												
Wasaga Beach 5	8 (6)	8.2 (6.1)	6	6.1	5	5.1	3	3.1	0	0	3	3.1
Wasaga Beach 1&2	4 (3)	6.6 (4.9)	4	6.6 (4.9)	5 (4)	8.2 (6.6)	3	4.9	3	4.9	5 (2)	8.2 (3.3)
New Wasaga	1	3.7	1	3.7	2	7.4	1 (0)	3.7 (0)	0	0	0	0
Totals	13 (10)	7.0 (5.4)	11 (10)	5.9 (5.4)	12 (11)	6.5 (5.9)	7 (6)	3.8 (3.2)	3	1.6	8 (5)	4.3 (2.7)
<u>Toronto Beaches</u>												
Breakwater	0	0	2	9.5	3	14.3	1	4.8	0	0	1	5.0
Hartlans Point	0	0	0	0	0	0	0	0	0	0	0	0
Kew Beach	0	0	0	0	0	0	0	0	0	0	0	0
Totals	0	0	2	6.9	3	10.3	1	3.4	0	0	1	3.4
<u>Hamilton Beaches</u>												
Confederation & Van Wagners	3	7.9	10	26.3	9 (7)	23.7 (18.4)	4	10.5	8 (6)	21.1 (15.8)	6	15.8
<u>Lake Erie Beaches</u>												
Long Point	10	7.3	3	2.2	8	5.8	2	1.5	26	19.0	6	4.4
Iroquois	7 (5)	10.4 (7.5)	4	6.0	5	7.5	2 (0)	3.0 (0)	5	7.5	2	3.0
Turkey Point	0	0	0	0	3	13.6	1	4.5	1	4.5	0	0
Totals	17 (15)	7.5 (6.6)	7	3.1	16	7.1	5 (3)	2.2 (1.3)	32	14.2	8	3.5
Grand Total	33 (28)	6.9 (5.8)	30 (29)	6.3 (6.1)	40 (37)	8.4 (7.7)	17 (14)	3.5 (2.9)	43 (41)	9.0 (8.6)	23 (20)	4.8 (4.2)

% - Percentage of Total Swimmer Respondents.
The figures in brackets are the numbers with symptoms with all dubious cases removed.

Table 16 - Illness Rates for Nonswimmers at the Ten Beaches

BEACH	No. of People	No. With Symptoms	Percentage
<u>Toronto Beaches</u>			
Breakwater	7	0	0 %
Hanlans Point	0	0	0 %
Kew Beach	2	0	0 %
Totals	9	0	0 %
<u>Hamilton Beaches</u>			
Confederation & Van Wagners Beach	6	2	33.3 %
<u>Lake Erie Beaches</u>			
Long Point	1	0	0 %
Turkey Point	3	1	33.3 %
Iroquois	8	1	12.5 %
Totals	12	2	16.7 %
<u>Wasaga Beach</u>			
Wasaga Beach 5	6	0	0 %
Wasaga Beach 1&2	4	0	0 %
New Wasaga	2	1	50 %
Totals	12	1	8.3 %
Grand Total	39	5	12.8 %

There were no dubious cases for nonswimmers.

Table 17 - Symptom Rates for Nonswimmers at the Ten Beaches

BEACH	No. with Gastro-intestinal Symptoms	%	No. with Respiratory Symptoms	%	No. with Ear, Eye, Nose Symptoms	%	No. with Allergic Symptoms	%	No. with Sunburn	%	No. with Other Symptoms	%
<u>Wasaga Beach</u>												
Wasaga Beach 5	0	0	0	0	0	0	0	0	0	0	0	0
Wasaga Beach 1&2	0	0	0	0	0	0	0	0	0	0	0	0
New Wasaga	1	50	0	0	0	0	0	0	0	0	0	0
Totals	1	8.3	0	0	0	0	0	0	0	0	0	0
<u>Toronto Beaches</u>												
Breakwater	0	0	0	0	0	0	0	0	0	0	0	0
Hanlans Point	0	0	0	0	0	0	0	0	0	0	0	0
Kew Beach	0	0	0	0	0	0	0	0	0	0	0	0
Totals	0	0	0	0	0	0	0	0	0	0	0	0
<u>Hamilton Beaches</u>												
Confederation & Van Wagners	0	0	2	33.3	0	0	0	0	0	0	1	16.7
<u>Lake Erie Beaches</u>												
Long Point	0	0	0	0	0	0	0	0	0	0	0	0
Iroquois	1	12.5	0	0	0	0	0	0	0	0	0	0
Turkey Point	0	0	0	0	0	0	1	33.3	0	0	0	0
Totals	1	8.3	0	0	0	0	1	8.3	0	0	0	0
Grand Total	2	5.1	2	5.1	0	0	1	2.6	0	0	1	2.6

% - Percentage of Total Nonswimmer Respondents.
There were no dubious cases for nonswimmers.

IPPERWASH CADET CAMP SURVEY

The types of illnesses experienced by the cadets during their stay at the camp may be seen in Table 18. The illness incidence was distributed as follows: upper respiratory tract infections 77%; gastrointestinal disorders 4.2%; skin infections 3.9%; eye infections 5.7%; and ear infections 9.2%. Illnesses due to colds were not included in the data. However, it should be stated that, according to the medical records, the common cold was the most predominant type of illness among the cadets during the summer.

The microbiological evaluations of the water and sediment samples taken from the swimming lesson sites are shown in Table 10. The geometric mean of fecal coliforms in water was shown to be 540 by MPN estimate and 150 by the MF technique. The geometric mean of fecal coliforms in sediment was 1600. Our results were corroborated by total coliform and fecal coliform counts of water samples collected on July 5 and August 1 by one of the camp staff members. The samples were analyzed by the London, Ontario, M.O.E. laboratory and the results reported as follows:

DATE	TOTAL COLIFORMS	FECAL COLIFORMS
	/100 mL	/100 mL
July 5, 1979	300	200
August 1, 1979	>160	>60

Table 18 - Incidence of various types of illness among cadets at Ipperwash Camp

Area of body Affected	Condition	Number of cases	% of total
Upper Respiratory Tract	Upper Respiratory Tract Infection (Sore Throat)	100	29.76
	Bronchitis	10	2.98
	Rhinopharyngitis (cough)	61	18.15
	Laryngitis	28	8.33
	Tonsillitis	57	16.96
	Sinusitis	3	0.89
Gastro-intestinal	Dyspepsia (sore stomach)	2	0.60
	Gastritis	2	0.60
	Diarrhea	2	0.60
	Nausea and Vomiting	7	2.08
	Nausea and Anorexia	1	0.30
Skin	Inflamitive Dermatosis	1	0.30
	Furuncles (boils)	6	1.79
	Infected Blisters	6	1.79
Eye	Conjunctivitis	8	2.38
	Stye (Staphylococcal)	11	3.27
Ear	Sore ear	9	2.68
	Chronic Earache	2	0.60
	Ear Cyst	1	0.30
	Post Ear Infection	1	0.30
	Bilateral Serous Otitis	1	0.30
	Otitis	3	0.89
	Otitis Media	7	2.08
	Otitis Externa	7	2.08

DISCUSSION

Relatively little research has been done on the incidence of disease associated with the use of fresh water beaches. However, much information concerning the etiological agents of swimming-related illnesses can be obtained from literature pertaining to epidemiological studies of swimming pools. For example, Hellerstrom⁽⁹⁾ reported skin nodules containing acid-fast bacilli occurring after small abrasions while bathing in swimming pools. In the U.S.A., Bell *et al.*⁽²⁾, investigating outbreaks of conjunctivitis in summer camps, found the incidence 50% greater among users of camp pools than among nonusers. The agent responsible for inclusion conjunctivitis, a member of the Chlamydia genus, evidently is initially derived from vaginal or urethral discharges of bathers and subsequently from conjunctival washings of those infected. Adenovirus infection has been associated with swimming in an unchlorinated pool⁽⁷⁾ and McLean⁽¹¹⁾ has isolated parainfluenza 1 virus from swimming pool water. Anderson and Jamieson⁽¹⁾ described a case of meningoencephalitis due to Naegleria fowleri in swimming pool water from which the organism was not eradicated by chlorination to 10 ppm. Serotyping and phage typing was used by Seyfried and Fraser⁽¹⁷⁾ to establish that Pseudomonas aeruginosa from swimming pool water was the causal agent of otitis externa ear infections in bathers. The organism has also been shown to cause a skin rash associated with the use of whirlpools⁽⁸⁾.

Ortiz⁽¹⁵⁾, in a study of fresh water ponds, used total and fecal coliforms plus fecal streptococci, Staphylococcus aureus, and Pseudomonas aeruginosa as indices of water pollution. Phage typing of the staphylococcal isolates from both the ponds and patients was used to demonstrate a relationship between pond swimming and upper respiratory infections.

Marine beaches were investigated by Brisou⁽³⁾ as a source of yeasts and fungi. He noted increased instances of vaginal infections caused by Candida among females using the marine beaches contaminated with intestinal fungi. Cabelli *et al.*⁽⁴⁾ also studied the impact of pollution on marine bathing beaches. They found that the rate of gastrointestinal symptoms increased at beaches with high Escherichia coli and enterococcus densities. Investigations of poliomyelitis among children living near the seashore in England and Wales prompted Moore⁽¹³⁾ to suggest that an increased rate of illness could be associated with swimming in marine waters that were defined as 'polluted' in terms of coliform densities.

A fresh water and tidal water epidemiological study outlined by Stevenson⁽¹⁹⁾ in 1953 compared illness rates among swimmers and nonswimmers at beaches on Lake Michigan; the Ohio River and a fresh water pool; and at tidal water beaches off Westchester County, N.Y. The results showed that there was an appreciably higher illness incidence in the swimming group and that eye, ear, nose, and throat ailments represented more than half the overall illness incidence, gastrointestinal disturbances up to one-fifth and skin irritations plus other illnesses, the balance.

As may be seen from the aforementioned reports, epidemiological evidence is accumulating that bathers using contaminated recreational water of any sort are at risk from cross-infection. Furthermore, numerous different pathogens may be implicated in a wide variety of swimming-related illnesses. The establishment of water quality guidelines is complicated by the fact that populations of potential pathogens in recreational waters may be unrelated to those of coliforms^(10,14). Also, diarrhea-producing bacteria such as Campylobacter fetus subsp. jejuni have not, as yet, been studied in natural bodies of water in Canada and their contribution to the incidence of swimming-related illness is unknown.

In this study we have attempted to establish the necessary criteria for an epidemiological survey of Great Lakes beaches. A variety of factors were found to influence the results of the study; one important environmental consideration was temperature. For example, on August 2 there was a 5°C difference in the water temperature at St. Catharines' Lakeside and Hamilton's Confederation Park. Even on the Labour Day weekend (September 1 to 3), the water temperature at the St. Catharines beaches was 3°C warmer than at the Hamilton beaches. Probably because of the cooler water temperature, people at the Hamilton beaches tended not to go swimming, as shown by the fact that during the survey there were a total of 541 people at the beach but only 3 of them were observed in the water. At the St. Catharines beaches, on the other hand, of the 217 people observed at the beach, 29 were active swimmers. It was also noted that a drop of 8°C in the air temperature at the Toronto Island beaches could bring about a corresponding drop in the number of people on the beach from over a thousand to zero. We can conclude from the observations that the air and water temperature at the beach should be at least 19°C in order to obtain a representative population of swimmers.

The direction and degree of wind also appears to be an important consideration in Great Lakes sampling. For example, a strong onshore wind may produce high waves and a consequent resuspension of the sediment organisms into the water column. As a result, the number of bacteria recovered from the surface water samples may be higher than expected. This phenomenon is often accompanied by a corresponding decrease in the number of sediment organisms one would normally expect to find.

Another environmental factor that should be taken into consideration is the amount of sunlight present on the day of sampling. It has been demonstrated previously that U.V. light may have a detrimental effect on the heterotrophic bacterial population in lake water. We found that, due to the effect of sunlight, counts of indicator organisms dropped significantly between 12:00 noon and 6:00 p.m. For this reason, on a bright sunny day, one should ensure that a proportion of the samples are collected in the morning and late afternoon.

Results of this study also suggest that both surface water and sediment samples should be analyzed to obtain a true picture of the microbiological quality at particular beach site. Because of the natural sedimentation process, bacterial indicators and small numbers of pathogens will settle to the bottom of the lake. These organisms may not always be prevalent in the surface water samples, as seen in our data for P. aeruginosa and S. aureus, but they could theoretically be resuspended from the sediment into the water column by the action of swimmers, waves, and boats. Carrying out a sediment sample analysis would ensure that all the indicator and pathogenic organisms at the site are properly accounted for.

The results presented in this report and summarized in Table 13 show clearly that bathing beaches on the Great Lakes may be differentiated using the levels of both bacterial indicators and potential pathogens such as P. aeruginosa, coagulase positive staphylococci, and Naegleria fowleri. The numbers of fecal coliforms at beaches on all three lakes ranged from minimal to >2400 in both water and sediment. If a geometric mean of 200 fecal coliforms/100 mL is used as a limit for surface water samples, Ipperwash on Lake Huron, and Selkirk and Port Dover on Lake Erie could be considered to have questionable beaches. Of the Lake Ontario beaches, Jones and Darlington Park were suspect in view of their fecal coliform counts. We also observed that, similar to the findings of Sherry et al.⁽¹⁸⁾, Lakeside Park on Lake Ontario had fecal coliform counts in the 10^3 /100 mL range.

Pseudomonas aeruginosa organisms were found to be present in the water and sediment at all beaches, regardless of the levels of fecal pollution. Nonetheless, Table 13 shows that the beaches high in fecal coliform and fecal streptococcus levels generally had high densities of staphylococci and Pseudomonas. Incidence of the pathogenic coagulase positive S. aureus and N. fowleri organisms did not appear to correspond with the highest fecal coliform levels at the beaches.

The results of the preliminary epidemiological survey could not be considered conclusive since there were only 518 participants. In addition, the number of swimmers was not balanced by an equal number of nonswimmers. However, useful information concerning the interviewing procedure was derived from this aspect of the study. For example, Questionnaire number two was handed out at the gates of selected Provincial Parks to determine if this would be an effective means of having one interviewer survey a large number of people. It was found that the return-rate of the questionnaires was very small, and unless a more official approach can be taken, this procedure will not be used in future studies.

During the interviewing procedure, an attempt was made to seek out family groups so that there would be a representative proportion of nonswimmers. Although this was not totally successful, it was found that the family groups tended to be more interested and they returned

their questionnaires more often than singles. The problem of obtaining enough nonswimmers for the survey may have to be resolved by utilizing Stevenson's⁽¹⁹⁾ method of canvassing non-swimmers of the same age and sex who are living close to the beach site area.

In this study, the return rate of the mail-in questionnaires ranged from 25 to 66%. An incomplete return of the follow-up questionnaires could inflate the illness rates, since a person with symptoms might be more likely to return a questionnaire than one without. A follow-up by telephone appears to be the best method of obtaining comprehensive data.

The questionnaire format should be reviewed and changed somewhat for future studies. Some of the problems experienced with the current forms were: (a) it was difficult to distinguish between respiratory illness and the symptoms of a runny or stuffed nose (these should probably both be included in the same category); and (b) there was no provision in the questionnaire for skin infections such as boils or rashes caused by organisms such as S. aureus and P. aeruginosa.

The survey of 1700 cadets at Camp Ipperwash presented a different approach to the problem of assessing swimming-related illnesses. Unless exempted for medical reasons, all cadets were required to participate in a one-hour swimming program each day. This meant that the cadets were exposed for the entire summer to bathing water that, according to the microbiological analyses (Table 13), was not ideal. At the end of the summer, the cadets' medical symptoms were tabulated and the distribution of illness was found to be as follows: upper respiratory tract infections; ear infections; eye infections; gastrointestinal disorders; skin infections.

There were a number of predisposing factors which may have been partially responsible for the onset of some of the infections. For example, the unusually cold weather during the early part of July probably contributed to the high incidence of coughs, colds, and sore throats. Laryngitis was a frequent complaint of cadets who were issuing commands on the parade square. Furthermore, blisters on the feet that subsequently became infected were, in most cases, caused by ill-fitting boots.

Although the fecal coliform recovery at Ipperwash Beach was in the same range as the numbers reported by Cabelli et al.⁽⁶⁾ for Coney Island Beach (i.e., 165/100 mL in 1973 and 565/100 mL in 1974), the incidence of gastrointestinal disorders among swimmers appeared to be higher at Coney Island. It should be noted however, that no direct comparisons can be made without the inclusion of a control group of nonswimmers at Ipperwash Beach.

In this cadet camp study, eye, ear, and skin infections accounted for a total of 18.8% of the illnesses. This data would tend to support the theory that the majority of infections acquired through bodily contact with recreational waters are associated with body surfaces or are upper respiratory in nature rather than gastrointestinal.

A site such as the Ipperwash Cadet Camp might be used in future for a more specific type of epidemiological survey. For example, the infirmary services at the camp would make it possible to collect swabs of any purulent discharges and to swab the ears of the cadets before they went in swimming. Subsequent serotyping and phage typing of isolates such as S. aureus, P. aeruginosa, Salmonella sp. and enteropathogenic E. coli collected from the bathing water and from fecal specimens and swabs of discharges would add much needed information to the documented data on water quality criteria.

STATISTICAL ANALYSIS OF SURVEY POPULATIONS

Calculations were performed to determine the number of participants required to demonstrate significant differences at given illness rates (Table 19).

When we are comparing swimmers and nonswimmers at one beach, the total number of people to be surveyed is twice the figure in the table, i.e., equally-sized groups of swimmers and nonswimmers. When we are comparing two beaches, the meaning of the table is slightly changed. $\bar{P}$ is the average rate for all swimmers and nonswimmers at both beaches. P_2 is the differential rate between swimmers and nonswimmers at the polluted beach, i.e., P_{Swimmers} at Polluted Beach $- P_{\text{Nonswimmers at Polluted Beach}}$. P_1 is the differential rate between swimmers and nonswimmers at the unpolluted beach, i.e., $P_{\text{Swimmers at Unpolluted Beach}} - P_{\text{Nonswimmers at Unpolluted Beach}}$. (An example will follow).

The total number of people surveyed should be four times the number in the table, four equally-sized groups of swimmers and nonswimmers at the two beaches.

NOTE: The basic formula for comparing two groups is derived by Fleiss in Statistical Methods for Rates and Proportions as:

$$n = \frac{(C\alpha/2 - C_1 - \beta)^2 \left(\sqrt{2\bar{P}\bar{Q}} - \sqrt{P_1Q_1 + P_2Q_2} \right)^2}{(P_2 - P_1)^2}, \text{ where } P_2 \geq P_1$$

For the low values of P_1 and P_2 to be used in this survey, we have also:

$$n \approx \frac{(C\alpha/2 - C_1 - \beta)^2 2\bar{P}\bar{Q}}{(P_2 - P_1)^2}$$

$$n \approx \frac{(C\alpha/2 - C_1 - \beta)^2 (P_1Q_1 + P_2Q_2)}{(P_2 - P_1)^2}$$

Table 19 - Size of groups to detect differences between two populations

$P_2 - P_1 = .01$	$\alpha = 0.05 \quad 1-\beta = 0.05$	$\alpha = 0.05 \quad 1-\beta = 0.5$
$P = .01$	~ 2 540	~ 750 (1114)
$P = .02$	~ 5 000	~ 1 500 (1685)
$P = .03$	~ 7 500	~ 2 250
$P = .04$	~ 10 000	~ 3 000
$P = .05$	~ 12 300	~ 3 640
$P = .10$	~ 24 000	~ 7 200
$P_2 - P_1 = .02$		
$P = .02$	~ 1 250	~ 370 (552)
$P = .03$	~ 1 875	~ 555 (682)
$P = .04$	~ 2 500	~ 740 (834)
$P = .05$	~ 3 100	~ 910 (988)
$P = .10$	~ 6 200	~ 1 800
$P_2 - P_1 = .03$		
$P = .03$	~ 850	~ 250 (371)
$P = .04$	~ 1 120	~ 333 (457)
$P = .05$	~ 1 380	~ 400 (476)
$P = .10$	~ 2 700	~ 800 (840)

P_1 = Proportion of population 1 with a given characteristic, (e.g., nonswimmers with a certain type of illness)

P_2 = Proportion of population 2 with the same characteristic, (e.g., swimmers with the same illness)

In calculating this table we assume $P_2 > P_1$:

α is the probability of inferring a difference in two populations when there is none, i.e., inferring that $P_2 \neq P_1$, when $P_1 = P_2$

$1 - \beta$ is the probability of missing a real difference between populations, i.e., inferring that $P_1 = P_2$ when $P_2 > P_1$

$P = \frac{P_1 + P_2}{2}$, if the groups are of equal size, or is a weighted average of P_1 and P_2 , if they are not.

The figures in brackets are group sizes incorporating the continuity correction. For small sample sizes, (under 1000), and low P , the effect is appreciable.

NOTE: on $1 - \beta = .05$ vs. $1 - \beta = .5$:

With $1 - \beta = .05$, the chances are nineteen in twenty of detecting any real differences in populations. With $1 - \beta = .5$, there is only one chance in two of detecting a real difference, or just as much chance of missing as detecting a real difference.

For comparing two beaches we have:

$$n \approx \frac{(C\alpha/2 - C_1 - \beta)^2 4\bar{P}\bar{Q}}{(P_2 - P_1)^2}, \text{ where ,}$$

as mentioned above, P_2 is the differential rate between swimmers and nonswimmers at the polluted beaches. P_1 is the differential rate between swimmers and nonswimmers at the unpolluted beach, and $\bar{P}$ is the average rate for all people at both beaches.

In actuality it is not possible to get exactly equally-sized groups, and a very high number of people surveyed in one group can reduce the number of people needed for the other group(s). Unless one group is several times the size indicated in the table, the effect is not great.

Examples to Show Use of Population Table

Example 1. A researcher is investigating whether swimmers are more likely to have chronic ear infections than nonswimmers. He knows that approximately 5% of the population in general has chronic ear infections, and wishes to detect whether at least 2% more swimmers than nonswimmers have chronic ear infections. His grant is large enough to make this a definitive study. How many swimmers and nonswimmers must be surveyed?

$$P = 0.05 \quad P_2 - P_1 = .02, \text{ where}$$

P is the proportion of the population in general with chronic ear infections.

P_2 is the proportion of swimmers with same.

P_1 is the proportion of nonswimmers with same.

For a definitive study, choose $1 - \beta = 0.05$, reducing the chance to one in twenty of not finding a real difference (of 2%), α is never more than 0.05. Then the number of each group, swimmers and nonswimmers is 3100.

Example 2. An experimenter is investigating two beaches, one polluted, one unpolluted. He hypothesizes that the effect of swimming at a clean beach will raise the probability of getting a particular symptom by about 2%, and that the effect of swimming at a polluted beach will raise the chance of getting the symptom by about 4%. He feels that the average symptom rate will be about 4%, for all people (swimmers and nonswimmers) at both beaches. How many people must be surveyed to test the hypothesis?

$$P_2 = .04$$

$$P_1 = .02$$

$$P_2 - P_1 = .02$$

$$P = .04$$

Choosing $\alpha = .05$, $\beta = .95$, (i.e., $1 - \beta = .05$). We find we need four groups of swimmers and nonswimmers at two beaches, each of size 2500 for a total of about ten thousand people.

DISCUSSION OF PROPOSED FUTURE SAMPLING SITES

1. WASAGA BEACH

Wasaga had the largest crowds of any of the surveyed beaches. It could be studied on at least one weekend when water pollution levels are high, and at least one when water pollution levels are low. The base rates for illness among nonswimmers might change from weekend to weekend, as various small ailments, such as colds or the flu, find their way around the general population. We are interested, however, not in the base rates, but in the difference in rates between swimmers and nonswimmers. A more serious objection is that there is no way of ensuring that the water quality will vary enough to show an appreciable change in the differential rate between swimmers and nonswimmers. None of our samples, six each from three beaches, indicated very high pollution. The highest fecal coliform count was under 300/100 mL water by membrane filtration, and 540/100 mL (followed by 240/100 mL) by the Most Probable Number technique (M.P.N.). Fecal streptococci counts varied from 0 to 52/100 mL water; staphylococci counts ranged from 2 to 181/100 mL water; and the highest Pseudomonas count was 13/100 mL water, obtained twice. The most polluted days had fecal coliform counts ranging around 200/100 mL water, low (except for one) Pseudomonas counts, low (<60/100 mL) fecal streptococci counts and staphylococci counts of about 150/100 mL water.

It would be possible to survey Wasaga throughout the summer, and try a regression analysis to tie the pollution indicator counts to the difference in illness rate between swimmers and nonswimmers.

2. CONFEDERATION PARK AND VAN WAGNERS BEACH

On a hot, sunny weekend, these beaches are packed with thousands of people. There are many nonswimmers who simply picnic, or lie in the sun. However, we did not survey a nearby beach that was polluted and extensively used, although there is a beach in Grimsby (not surveyed). Comparing Confederation Park against itself on two weekends entails the same risks as comparing Wasaga Beach against itself. The variation in microbiological readings was quite large. On Sunday August 12, a clear sunny day, the fecal coliform count was 1600/100 mL by M.P.N. and too numerous to count (TNTC) by membrane filtration. Pseudomonas aeruginosa levels were 23/100 mL water and 23/100 gm sediment. Fecal streptococci and staphylococci were both TNTC. On Sunday, September 9, a cloudy day, fecal coliform counts were 2/100 mL water by M.P.N. and 12/100 mL water by membrane filtration; the fecal streptococcal and staphylococcal counts were 2/100 mL and 6/100 mL, respectively.

One of the uncertainties about surveying Confederation Park is the weather. The water is cold, and few people go in swimming unless the day is very hot and sunny.

3. ST. CATHARINES BEACHES (LAKESIDE, (WELLER) MUNICIPAL, AND JONES))

These beaches are near each other. The pollution levels are erratic, and during the course of a summer both high and low levels of pollution can occur. The Canada Inland Waters Survey (1977),⁽¹⁸⁾ found Lakeside more polluted in July 1977, and Municipal (Weller) Park more polluted in August, of the same year. The bather load was given as 200-300 at Weller (Municipal Park), and 300-400 at Lakeside on Monday July 4 (an American holiday).

There are two samples from each of Lakeside and Jones (close to Weller, on the other side of the canal, and possibly microbiologically close) on the same day. The fecal coliform count in the water was high at Jones and low at Lakeside on August 2; on October 10, the situation was reversed. Other indicators (particularly the fecal coliform sediment count, which was high at Jones on October 10 and low on August 2, and the reverse at Lakeside) did not follow such a convenient pattern.

A survey here would probably have to be done over several weekends to get a large enough sample population. A close monitoring of pollution levels is necessary. The statistical analysis will be less straightforward if the pollution levels are constantly changing.

Some further microbiological testing should be done this Spring.

4. SELKIRK PROVINCIAL PARK vs LONG POINT or TURKEY POINT PROVINCIAL PARK

These are three provincial parks on Lake Erie. Selkirk was, after Port Dover, the most polluted beach on Lake Erie. Turkey Point and Long Point are nearby (within 80 km) provincial parks with much lower pollution levels. All three parks have campers and weekend visitors as well.

From the microbiological data, of the two days when all three beaches were surveyed, it appears that Turkey Point was more "in sync" with Selkirk than Long Point, in the sense that the microbiological indicators rose and lowered together. This could be due to the time of day of sampling or other factors. Further tests could be done before the swimming season starts to confirm (or refute) this.

The water is warm at all three beaches.

These beaches are worth surveying. Further microbiological tests should be done in Spring.

5. MUNICIPAL (WELLER) PARK AND HOLIDAY BEACH

These two beaches are located on Lake Ontario and Lake Erie respectively. Background information on Municipal Park is available from the report published by Sherry *et al.*⁽¹⁸⁾. Holiday Beach is a Provincial Park, with campsites, near Windsor. These two sites are of interest because of the isolation of N. fowleri from the water. Further studies will be carried out to assess the microbiological quality of the sewage treatment plant effluent discharged near the beaches.

REFERENCES

1. Anderson, K. and A. Jamieson. 1972. Primary amoebic meningoencephalitis. *Lancet* i: 902-903.
2. Bell, J.A., W.P. Rowe, J.T. Engler, R.H. Parrott, and R.J. Huebner. 1955. Pharyngoconjunctival fever. Epidemiological studies of a recently recognized disease entity. *Jour. Amer. Med. Assoc.* 157: 1083.
3. Brisou, J. 1975. Yeasts and fungi in marine environments. *Société française mycologie médicale, Bulletin*, 4: 159-162.
4. Cabelli, V.J., A.P. Dufour, M.A. Levin, and P.W. Habermann. 1976. The impact of pollution on marine bathing beaches: An epidemiological study. In: *Middle Atlantic Continental Shelf and the New York Bight*. *Limnol. Oceanogr.* 2: 424-432.
5. Cabelli, V.J., M.A. Levin, A.P. Dufour and L.J. McCabe. 1975. The development of criteria for recreational waters. In *Discharge of Sewage from Sea Outfalls*, Ed. by A.L.H. Gameson, Pergamon, London: 63-73.
6. Dutka, B.J. and J.B. Bell. 1973. Isolation of *Salmonellae* from moderately polluted waters. *J. Water Pollut. Control Fed.* 45: 316-324.
7. Foy, H.M., M.K. Cooney, and J.B. Hatlen. 1968. Adenovirus Type 3 epidemic associated with intermittent chlorination of a swimming pool. *Archives Environ. Health* 17:795.
8. Health and Welfare Canada. 1979. Rash associated with use of whirlpools. *Canada Diseases Weekly Report* :5-45, 205-206.
9. Hellerstrom, S. 1951. Collected cases of inoculation lupus vulgaris. *Acta. Dermato-Venereologica* 31: 194.
10. Kush, B.J. and A.W. Hoadley. 1977. The association of *Pseudomonas aeruginosa* with commercial whirlpool waters. *Abstr. Annu. Meet. Am. Soc. Microbiol.* 271.
11. McLean, D.M. 1963. Infection hazards in swimming pools. *Pediatrics*, Springfield 31: 811.
12. Mechalas, B.J., K.K. Hekimian, L.A. Schniazi, and R.H. Dudley. 1972. An investigation into recreational water quality. *Water quality criteria data book*, Vol. 4. 18040 DAZ 04/72, U.S. EPA.
13. Moore, B. 1959. Sewage contamination of coastal bathing waters in England and Wales. A bacteriological and epidemiological study. *J. Hyg., Camb.* 57: 435-472.

14. Nemedi, L. and B. Lanyi. 1971. Incidence and hygienic importance of Pseudomonas aeruginosa in water. Acta. Microbiol. Acad. Sci. Hung. 18: 319-326.
15. Ortiz, J.S. 1977. The use of Staphylococcus aureus as an indicator of bather's pollution. Abstr. Annu. Meet. Am. Soc. Microbiol. 271.
16. Seyfried, P.L. (Unpublished data).
17. Seyfried, P.L. and D.J. Fraser. 1978. Pseudomonas aeruginosa in swimming pools related to the incidence of otitis externa infection. Health Lab. Sci. 15: 50-57.
18. Sherry, J.P., S.R. Kuchma, J. Zarzour and B.J. Dutka. 1979. Occurrence and significance of Candida albicans in Lake Ontario bathing beaches. Inland Waters Directorate, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, Scientific Series No. 98, 1-31.
19. Stevenson, A.H. 1953. Studies on bathing water quality and health. Am. J. Publ. Hlth. 43: 529-538.
20. U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. USEPA, Washington, D.C. 42-50.

APPENDIX A

PATHOGENIC AMOEBAE

In order to protect water recreationists from water and sand-borne infectious agents, Ontario waters have been monitored for bacteria of fecal origin. Recently it has become apparent that several groups of microorganisms and viruses should be included in any major examination of water which may pose a potential health hazard. Enteric viruses and pathogenic fungi are beginning to be included in environmental surveillance programs (Sherry et al, 1979). Another group of microorganisms which should be included in surveillance programs is the so-called free-living soil amoebae. The Associate Committee on Scientific Criteria for Environmental Quality of the National Research Council Canada has stated that "further research is necessary to determine whether Naegleria and Acanthamoeba sp. constitute a hazard in heated waters in Canada" (Dickson, 1975).

Members of the superclass Sarcodina (amoebae) are usually divided into two groups according to their habitats (Kudo, 1963). The first group is free-living amoebae which multiply at the expense of bacteria and other small organisms. The second group is usually found in some type of association with other eucaryotic organisms; the relationship usually varies from commensalism to parasitism (Kudo, 1963). It was, therefore, surprising to some students of the Sarcodina that the so-called free-living group included members which were pathogenic under certain conditions. The infection of man by free-living amoebae was predicted several years before actual cases of the disease were recognized (Culbertson, 1961). It was Culbertson et al (1958) who first isolated a free-living amoeba from the culture fluid of growing trypsinized monkey-kidney cells thought to contain an unknown virus. Injection of the culture fluid into mice and monkeys had previously resulted in death of the animals with the production of brain lesions. The lesions were found to contain only the free-living amoebae identified as Acanthamoeba sp. Intracerebral injections or intranasal instillation into a second set of animals using pure cultures of the isolate resulted in the death of the animals from primary meningoencephalitis (Culbertson et al, 1958). A careful review of the research literature then indicated that free-living amoebae may have been involved in human disease (Derrick, 1948; Kernohan et al, 1960). In 1966, the first isolations of free-living amoebae from fatal cases of amoebic meningoencephalitis were accomplished (Butt et al, 1968; Carter, 1968; Culbertson et al, 1968). The causative agent of human meningoencephalitis proved to be an amoeboflagellate of the genus Naegleria (Butt et al, 1968).

Pathogenic Naegleria sp. may exist as typical amoebic trophozoites or they may be found as biflagellate swimming cells; trophozoites may encyst under certain conditions (Culbertson, 1971). Occasional reports of human meningoencephalitis caused by other sarcodines have appeared in the literature but there is no firm evidence linking organisms other than Naegleria fowleri with fatal disease (Culbertson, 1971). Human amoebic meningoencephalitis is a worldwide disease

since confirmed cases have been reported from California, Florida, Georgia, Texas, and Virginia, as well as Australia, Belgium, Czechoslovakia, England, India, New Zealand and Uganda (Carter, 1972; Culbertson, 1971; Singh and Das, 1972b). Additional cases have come to light by retrospective diagnosis of tissue sections.

Free-living amoebae are ubiquitous in soil and environments throughout the world (Kudo, 1963). The ease with which sarcodines may be isolated from the natural habitat undoubtedly has resulted in an underestimation of their pathogenic potential. The usual method of isolation for the past 30 years consisted of placing samples of mud or water on nonnutrient agar seeded with a thin lawn of Escherichia coli (Singh, 1941). The moist plates were then incubated at room temperature or at 37°C to allow multiplication of the phagotrophs. Recently, it was shown that room temperature or 37°C incubation allowed nonpathogenic sarcodines to outgrow the more virulent Naegleria sp. (Griffin, 1972). Thus, this overgrowth has resulted in an underestimation of the number of potentially pathogenic species present in mixed inocula (Griffin, 1972). Incubation of mixed inocula at 43°C allows the selective growth of virulent Naegleria fowleri (aerobia). The highest temperature tolerated by strains of N. fowleri (aerobia) is 46°C which is well above the temperature of human fever (Griffin, 1972). Acanthamoeba sp. with the ability to grow at 47°C have been isolated by W.D. O'Dell (personal communication). Both Naegleria sp. and Acanthamoeba sp. were isolated from laboratory waterbaths at the University of Hawaii which were daily raised to 45°C (Cotter, unpublished observations). Similar contamination of oyster tissue sections was traced to a laboratory waterbath held at 42°C (Sawyer and Buchanan, 1971). These latter authors stressed the need for regular cleaning of waterbaths to avoid any health hazard. Contamination of laboratory waterbaths as well as "backyard" pools may be the results of airborne cysts since 39 different strains of moderately pathogenic amoebae were isolated from air samples in England (Kingston and Warhurst, 1969). Chlorination does not seem to be effective against N. fowleri since a case was reported from Australia in which trophozoites were not destroyed in a swimming pool even after superchlorination (Anderson and Jamieson, 1972).

A link between human fecal matter and the selective growth of pathogenic free-living amoebae was amply demonstrated in studies from India by Singh and Das (1972a). These workers isolated three different species of pathogenic free-living amoebae from five separate localities known to contain sewage sludge in Lucknow, India. More frightening for those in industrialized societies is the possibility that thermal pollution, combined with fecal pollution, might result in the selection of the more virulent Naegleria sp. by elimination of the low-temperature tolerant, nonpathogenic species (Griffin, 1972). Ecological studies are underway in many areas of the world to evaluate this possibility. The authors have recently isolated high-temperature tolerant,

free-living amoebae from a "fecal based" ecosystem in southeastern Massachusetts. It should be noted that the majority of fatal amoebic meningoencephalitis cases occurred in the summer and were associated with swimming in nonsaline environments (Culbertson, 1971).

It is possible that Naegleria sp. may be more pathogenic for man and animals than Acanthamoeba sp. because the former has the ability to transform into a biflagellated swimming stage in hypotonic environments (Singh and Das, 1972b). The swimming stage is thought to enter the nasal passages of man and then to migrate to the brain via the olfactory bulbs (Carter, 1968; Culbertson, 1971). The resulting meningoencephalitis resembles some acute bacterial meningitis. Although chemotherapy is generally ineffective against the disease, a recent report suggests that immunotherapy may be developed (Thong et al, 1978).

With the above information in mind, it might be expected that Canadian waters would normally be relatively free of pathogenic varieties of Naegleria sp. However, increasing use of heated swimming pools as well as the use of bodies of water for thermal discharge raises the possibility of severe naeglerial contamination of recreational waters. Due to the input of electric generating stations, steel mills and municipal waste treatment plants, the present thermal input of 9.98×10^{10} BTU per hour to the total Great Lakes system is expected to increase more than eleven-fold to 114×10^{10} BTU per hour by the year 2000 (Denison and Elder, 1970).

METHODS

A new technique for the isolation of Naegleria and Acanthamoeba species has been developed which seems to offer advantages over previously established techniques (Chang, 1971). The technique is based on the following:

1. The use of an agar medium containing 1 gram lactose, 1 gram Bacto peptone, and 15 grams Bacto agar suspended in 1 litre of potassium phosphate buffer (5 mM) at pH 7.0.
2. The host bacterium used to culture amoebae is Escherichia coli strain B/r (ATTC 12407). The choice of the above bacterium and medium is based on the following criteria:
 - (a) The low ionic strength of the medium allows amoebae excystment and delays later encystment.
 - (b) The lactose allows selective growth of the host bacterium at high temperature while suppressing growth of bacteria introduced in the test samples.
 - (c) The ability of the host bacterium to grow above 44°C.
 - (d) The lack of filament and capsule formation by E. coli B/r.

The maximum temperature tolerance tests have been carefully conducted since high temperature may select for the overgrowth of pathogenic variants of Naegleria fowleri (Griffin, 1972). In spite of the occurrence of possibly negative mouse inhalation tests, it is widely held that the isolation of Naegleria fowleri capable of growing at 45°C indicates that pathogenic varieties of N. fowleri are probably also present in the same samples (DeJonckheere, 1977).

RESULTS AND DISCUSSION

Table 1 indicates the maximum growth temperature for amoebae initially isolated at 42°C. The amoebae in cultures number 1A collected on September 9 at Weller Park and the amoebae collected on September 10 at Holiday Beach were capable of good growth at 46°C; in addition, these amoebae were capable of making the transition from amoebae to flagellates when suspended in distilled water. Mouse inhalation tests were positive for these cultures, i.e., the mice were killed.

The conclusion is that samples obtained from Weller Park and Holiday Beach contain N. fowleri. The two recreational areas (Weller Park and Holiday Beach) should be closely monitored this summer for total Naegleria counts so that an estimate of their prevalence and distribution can be made.

Table 1 - Maximum Growth Temperature on 0.1% Lactose Peptone 5 mM KPO₄ Plates Seeded with E. coli B/r.

Collection Date	Original Sample Number	Location	Growth at 42°C	Growth at 43°C	Growth at 44.5°C	Growth at 46°C
August 15, 1979	No. 2 water	Darlington Provincial Park, Group Picnic Area	+	+	-	-
August 18, 1979	No. 17 water	Hanlans Point Central Island, Toronto	+	-	-	-
August 23, 1979	No. 3 water	Rondeau Provincial Park	+	+	-	-
August 23, 1979	No. 7 water	Turkey Point	+	-	-	-
August 25, 1979	No. 10 water	Hanlans point	+	+	+	-*
September 8, 1979	No. 1 water	Wasaga Beach	+	-	-	-
September 8, 1979	No. 2 water	Wasaga Beach	+	+	+	-*
September 8, 1979	No. 6 water	Hanlans Point	+	-	-	-
September 9, 1979	No. 1A water	Weller Park ^(a)	+	+	+	+
September 9, 1979	No. 3A water	Lakeside Court	+	-	-	-
September 10, 1979	--	Holiday Beach collected by Univ. Windsor	+	+	+	+
September 10, 1979	--	Mersea Beach collected by Univ. Windsor	+	+	+	-*

Symbols: + good growth; ± fair growth; - no growth; * cultures selected for mouse inhalation tests.

(a) Municipal Park (of City of St. Catharines)

REFERENCES

- Anderson, K. and A. Jamieson. 1972. Primary amoebic meningoencephalitis. *Lancet* 1972 Number 7756: 902-903.
- Butt, C.G., C. Baro, and R.W., Knorr. 1968. Naegleria (sp.) identified in amoebic encephalitis. *American J. Clin. Path.* 50: 568-574.
- Carter, R.F., 1968. Primary amoebic meningoencephalitis: Clinical, pathological, and epidemiological features of six fatal cases. *J. Path. Bacteriol.* 96: 1-25.
- Carter, R.F., 1972. Amoebic meningoencephalitis. *Trans. Roy. Soc. Trop. Med. and Hyg.* 66: 193-213.
- Chang, S.L. 1971. Small, free-living amoebas: cultivation, quantitation, identification, classification, pathogenesis, and resistance, p. 201-254. *In* T.C. Cheng (ed.), *Current Topics in Comparative Pathobiology*. Volume 1. Academic Press, New York.
- Culbertson, C.G., 1961. Pathogenic Acanthamoeba (Hartmanella). *Amer. J. Clin. Path.* 37: 195-202.
- Culbertson, C.G., 1971. The pathogenicity of soil amoebas. *Ann. Rev. Microbiol.* 25: 231-254.
- Culbertson, C.G., P.W., Ensminger, and W.M., Overton. 1968. Pathogenic Naegleria sp. - Study of a strain isolated from human cerebrospinal fluid. *J. Protozool.* 15: 353-363.
- Culbertson, C.G., J.W., Smith, and J.R., Minner. 1958. Acanthamoeba: Observations on animal pathogenicity. *Science* 127: 1506.
- DeJonckheere, J.F., 1977. Use of an axenic medium for differentiation between pathogenic and nonpathogenic Naegleria fowleri isolates. *Appl. Environ. Microbiol.* 33: 751-757.
- Denison, P.J., and F.J., Elder. 1970. Thermal inputs to the Great Lakes 1968-2000. *Proc. 13th Conf. Great Lakes Res.*, pp. 811-828.
- Derrick, E.H., 1948. A fatal case of generalized amoebiasis due to a protozoan closely resembling, if not identical with Iodamoeba butschlii. *Trans. Roy. Soc. Trop. Med. and Hyg.* 42: 191-198.
- Dickson, D.R., 1975. Waste heat in the aquatic environment. National Research Council of Canada. NRCC No. 14109, 30 pgs.
- Griffin, J.L., 1972. Temperature tolerance of pathogenic and nonpathogenic free-living amoebas. *Science* 178: 869-870.

- Heal, O.W., 1971. Protozoa, pp. 51-71. In I.B.P. Handbook No. 18. Methods of Study in Quantitative Soil Ecology: Population, production and energy flow. J. Phillipson (ed.). Blackwell Scientific Publications.
- Kernohan, J.W., T.B. Magath, and G.T. Schloss, 1960. Granuloma of brain probably due to Endolimax williamsi (Iodamoeba burshlii). A.M.A. Arch. Path. 70: 570.
- Kingston, D. and D.C. Warhurst, 1969. Isolation of amoebae from the air, J. Med. Microbiol. 2: 27-36.
- Kudo, R.R. 1963. Protozoology, 4th Ed., Charles C. Thomas, Springfield, Ill., pp. 20-38.
- Sawyer, T.K., and L.R., Buchanan, 1971. Contamination of tissue sections of the American oysters by cysts of Acanthamoeba sp. J. Invert. Path. 18: 300.
- Sherry, J.P. S.R., Kuchma, and B.J. Dutka, 1979. Confirmation tests for freshwater isolates of Candida albicans. Canadian Research. 12: 33-36.
- Singh, B.N., 1941. Selectivity in bacterial food by soil amoebae in pure mixed culture and in sterilized soil. Ann. Appl. Biol., 28: 52-53.
- Sing, B.N., and S.R., Das, 1972a. Occurrence of pathogenic Naegleria aerobia, Hartmannella culbertsoni and H. rhyodes in sewage sludge samples of Lucknow. Curr. Sci. 41: 277-281.
- Singh, B.N. and S.R., Das, 1972b. Intra-nasal infection of mice with flagellate stage of Naegleria aerobia and its bearing on the epidemiology of human meningoencephalitis. Curr. Sci. 41: 625-628.
- Thong, Y.H., C., Shepherd, A., Ferrange, and B., Rowan-Kelly, 1978. Protective immunity to Naegleria fowleri in experimental amoebic meningoencephalitis. Amer. J. Trop. Med. Hyg. 27: 238-240.

APPENDIX B

VIRUSES

Table of Contents

	<u>Page</u>
INTRODUCTION.....	87
MATERIALS AND METHODS.....	91
RESULTS	97
DISCUSSION	103
SUMMARY.....	104
REFERENCES.....	105

List of Tables

1. Human Enteric Viruses That May Be Present In Water	88
2. Recovery of Poliovirus from 40 L Volumes of Water by Duofine Membrane Filtration	99
3. Collection of Water and Sediment Samples	100
4. Virological Analyses of Lake Water and Sediment Samples	100
5. Virological Analyses of Sewage and Receiving River Water Samples.....	101

List of Figures

1. Possible modes of enteric virus transmission by water	89
2. Photograph of the Duofine membrane filtration system set up at a bathing beach site.....	93
3. Electron micrograph of a cluster of negatively stained enteroviruses	102
4. Electron micrograph of a cluster of negatively stained reoviruses.....	102

INTRODUCTION

Over 100 types of enteric viruses are known to be excreted in human feces. Table 1 lists the major groups of enteric viruses which have been isolated from raw sewage or feces of infected persons. As many as 10^9 virus particles may be excreted per gram of feces and concentrations as high as 500 000 infectious particles per litre of raw sewage have been found^(1,9). However, the amount of virus present in raw sewage at any given time is highly variable. Peak levels usually occur in late summer and early fall and lower densities can be found during the rest of the year^(1,2,3,4).

Enteric viruses easily survive secondary sewage treatment and chlorination, as commonly practiced, and thereby are discharged into natural waters where they can persist for many months. Since enteric viruses are considerably more resistant to various sewage and water treatment methods than either coliform or enteropathogenic bacteria, the absence of the latter organisms may not guarantee the absence of a viral disease hazard. Several studies conducted in developed countries have demonstrated enteric viruses in sewage effluents, contaminated streams, rivers, lakes, and drinking water^(1,5,6,7,8). Such water is considered a health hazard when it is used for drinking, recreational or irrigational purposes. Seafood from contaminated waters may also carry infectious viruses. Figure 1 illustrates some of the routes by which viruses from human and animal wastes may find their way to susceptible human hosts.

Enteric viruses are known to be responsible for numerous waterborne outbreaks of disease. Waterborne outbreaks caused by hepatitis A virus are well documented^(1,10) and adenovirus infections among persons frequenting swimming pools have been recognized. Recently, coxsackievirus B5 was isolated from an open lake swimming area during an outbreak of the virus at a summer boys' camp^(1,8). However, little direct evidence exists, at present, for the waterborne transmission of other enteric viruses to bathers. This is due to a lack of epidemiological studies and limitations of methods for detecting viruses in the environment.

Various problems have been encountered in conducting viral epidemiological studies. Many enteric viruses cause inapparent infections which are difficult to recognize as waterborne. Even if the infections are apparent, the broad spectrum of disease syndromes caused by most enteric viruses would make it difficult to attribute scattered cases of illness to a single etiological agent⁽¹⁾. In addition, suitable in vitro methods for the isolation of some enteric viruses (e.g., hepatitis A virus and rotaviruses) are presently not available. Furthermore, viruses in water are usually present in very small quantities which in many instances, may be beyond the detection limits of currently available methods. As little as one plaque forming unit (PFU) of virus reported to be capable, under proper circumstances, of producing infection in man^(1,9,11,12,13). Therefore, the presence of even one detectable infectious virus unit in water poses a potential disease hazard⁽¹⁾.

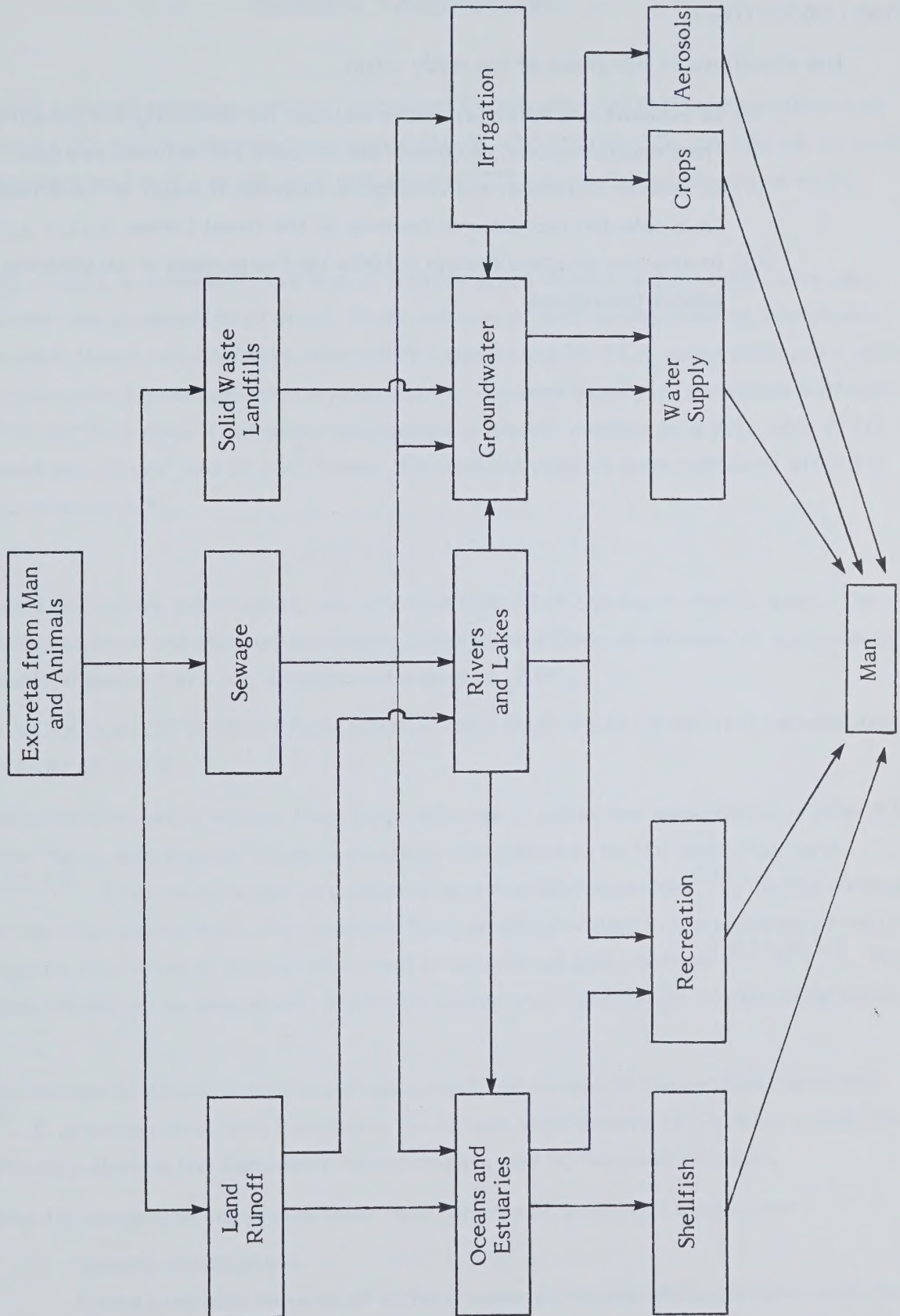
Table 1^(a) - Human Enteric Viruses That May Be Present In Water^(b)

Virus Group	Number of types	Disease caused
Enteroviruses		
Poliovirus	3	Paralysis, meningitis, fever
Echovirus	34	Meningitis, respiratory disease, rash, diarrhea, fever
Coxsackie virus A	24	Herpangina, respiratory disease, meningitis, fever
Coxsackie virus B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enterovirus types 68-71	4	Meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis, fever
Hepatitis Type A (probably an enterovirus)	1	Infectious hepatitis
Gastroenteritis Type A (probably an enterovirus)	2	Epidemic vomiting and diarrhea, fever
Rotavirus (Reovirus family)	≥ 1	Epidemic vomiting and diarrhea, chiefly of children
Gastroenteritis Type B		
Reovirus	3	Not clearly established
Adenovirus	≥ 30	Respiratory disease, eye infections

a. Taken from Melnick⁽⁹⁾.

b. Other stable viruses that might contaminate water are; SV40-like papovaviruses, which appear in the urine. The JC subtype is associated with progressive multifocal leuko-encephalopathy. Creutzfeld-Jakob disease virus. Like scrapie virus, the C-J virus resists heat and formaldehyde. The virus causes a spongiform encephalopathy, characterized by severe progressive dementia and ataxia. Parvoviruses. The adeno-associated satellite viruses that are excreted in stools of children survive heating for one hour at 60°C.

Figure 1 - Possible modes of enteric virus transmission by water⁽⁹⁾



PHASE I OBJECTIVES

The objectives of this phase of the study were:

- (i) to evaluate and refine available methods for detecting and isolating viruses from natural waters, sediments and influent and effluent sewage;
- (ii) to initiate collection and virological analyses of water and sediment samples from selected recreational beaches on the Great Lakes;
- (iii) to examine selected sewage outfalls for the purpose of establishing virological techniques.

MATERIALS AND METHODS

A. MEDIA

Eagle's minimal essential medium containing Earle's salts (MEM E) and supplemented with gentamicin (50 $\mu\text{g/mL}$), sodium bicarbonate (11 mL of 5.6% NaHCO_3 per 500 mL of medium), L-glutamine (2mM/mL) and 5 or 2% fetal calf serum (FCS) was used throughout this study.

B. CELL CULTURES

BS-C-1 cells, a continuous cell line of African green monkey kidney cells, were used for the isolation and propagation of virus. Stock cultures of cells were grown as monolayers in 75 cm^2 plastic tissue culture flasks using MEM E containing 5% FCS, gentamicin and L-glutamine. The cells were trypsinized with trypsin (0.25%) -versene (0.05%) in phosphate buffered saline at 37°C for 5-15 min. They were resuspended in growth medium at a split ratio of 1:3 and inoculated into 75 cm^2 and 25 cm^2 flasks. The cell monolayers were confluent after 3-4 days of incubation at 37°C.

C. VIRUS

Type 1 poliovirus, Sabin strain, was employed for all of the experimental work. The virus was obtained from the Ministry of Health, Laboratories Services Branch. It was passaged in BS-C-1 cells, dispensed in 5 mL aliquots and stored at -70°C.

D. CONCENTRATION OF VIRUS FROM WATER AND WASTEWATER BY THE MEMBRANE FILTRATION METHOD

The concentration of viruses from large volumes of water and wastewater by adsorption to and elution from microporous filters is presently considered to be the most promising method^(14,15,17). It has been tentatively adopted as a standard procedure⁽¹⁶⁾. In this method, viruses are adsorbed electrostatically to filters from acidified water, in the presence of cations, and subsequently recovered by elution with smaller volumes of basic buffers^(14,15,16,17). The concentrated viruses in the final eluate are then isolated and identified by conventional procedures.

The method employed in this study was a modified version of the proposed standard method⁽¹⁶⁾. It is broken down into 5 sections: (i) Sample conditioning; (ii) Primary concentration; (iii) Primary elution; (iv) Secondary concentration; and (v) Secondary elution.

1. Procedure for concentrating viruses from field samples of water and wastewater

(i) Sample conditioning.

Forty litre grab samples of surface water or sewage effluents were collected in 2 x 20 L collapsible, plastic containers which were used only once to avoid

cross-contamination of samples. In order to enhance adsorption of virus, Earle's balanced salt solution (EBSS) which acted as a source of cations, was added to each sample to give a final concentration of 1:100 and the pH of the sample was adjusted to 5.5-6.0 with 1N hydrochloric acid (HCl). Sewage effluent samples were treated, in addition, with 0.5% sodium thiosulphate to a final concentration of 1:100, in order to remove free chlorine.

(ii) Primary concentration.

The filtration system was set up as shown in Figure 2. The conditioned water was filtered under gravity, through 2 melamine-fiberglass pleated membrane filters (Duofine series, Filterite Corp. Timonium, Md.): a 3.0 μm porosity filter which served as a prefilter and 0.45 μm filter. When all of the sample had passed through the filters, the filter housings were disconnected from the reservoir and the remaining fluid inside the filter housings was poured out. The outlets were sealed with parafilm and the filter housings were placed on ice packs for transportation to the laboratory where they were stored overnight at 4°C.

The collection, conditioning and primary concentration of samples were generally carried out in the field. However, in poor weather conditions, the water samples were chilled on ice and brought back to the laboratory for complete processing.

(iii) Primary elution.

Residual water in the filter housings was removed and discarded by applying air pressure. Adsorbed virus was eluted from the filters by passing 800 mL of freshly prepared 0.05 M glycine buffer (containing 0.0005% phenol red and adjusted to pH 11.5 by the addition of 10 N sodium hydroxide (NaOH) through the 3.0 μm prefilter and then the 0.45 μm filter. The pH of the eluate was measured after passing through the first filter to ensure that no significant drop in pH had occurred. If the pH dropped below 11.0, then 1 N NaOH was used to adjust it back to 11.5 before passing the glycine buffer through the second filter. The glycine was permitted to remain in contact with the filters for about 1-2 mins., before being forced out of the filter housings with positive pressure. The eluate was neutralized with 0.05 M glycine (adjusted to pH 1.0 with 12 N HCl) immediately after collection because of the sensitivity of viruses to inactivation at high pH.

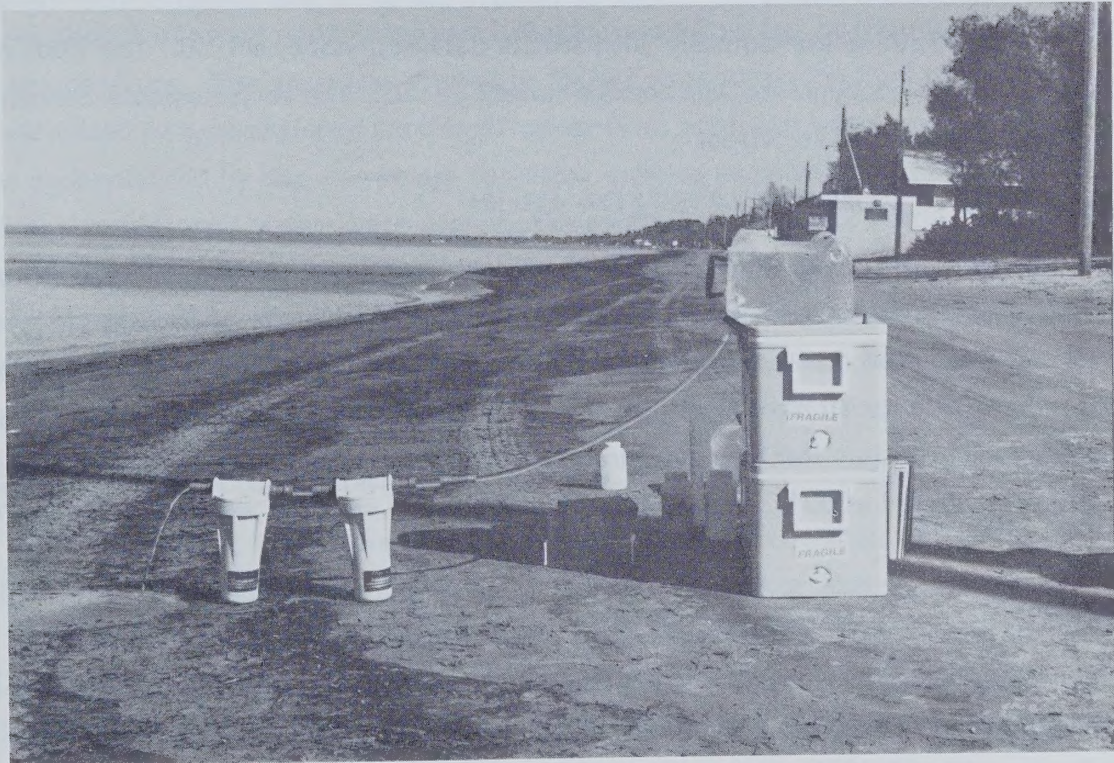


Figure 2 - Photograph of the Duofine membrane filtration system set up at a bathing beach site

(iv) Secondary concentration.

In order to enhance virus adsorption during reconcentration, the pH of the primary eluate was lowered to 3.5 with 0.05 M glycine, pH 1.0 and 0.05 M aluminum chloride (AlCl_3) was added to a final concentration of 0.0005 M. The eluate was filtered under vacuum through a layer consisting of, from top to bottom, a teaspoon of Celite 503 filter aid (J.T Baker), a 47 mm diameter AP 25 prefilter disc (Millipore Corp.) and a 47 mm, cellulose nitrate membrane (0.45 μm porosity, Millipore Corp.). After filtration, the filters were washed with 30 mL of 0.15 M sodium chloride (NaCl), pH 3.5, to remove residual AlCl_3 .

(v) Secondary Elution.

The virus was eluted from the filters with 8 mL of 0.05 M glycine, pH 11.5 containing 2% FCS. The eluate was passed through a 25 mm, 0.45 μm porosity membrane (Millipore, Corp.) to remove bacterial and fungal contaminants and neutralized immediately with sterile 0.05 M glycine, pH 1.0. The final volume of the concentrate was approximately 10 mL. The entire sample-concentrate was assayed for virus.

E. ASSAY OF SAMPLE-CONCENTRATES FOR VIRUS

The growth medium was removed from confluent monolayers of BS-C-1 cells grown in 25 cm² flasks. Each cell culture flask was then inoculated with 1 mL of the sample. Ten replicae cultures were used for each sample to be assayed for virus. The inoculated cultures were incubated at 37°C for 60 min. to allow virus adsorption to occur. At the end of this period, 5 mL of maintenance medium (MEM E containing 2% FCS) was added to each flask and the cell cultures were reincubated at 37°C. Uninoculated cell culture controls were included in the assay.

1. First Passage

The cell cultures were observed microscopically for viral cytopathic effects (CPE); daily for the first 3 days and then periodically for a total of 14 days. Cell cultures exhibiting CPE were frozen when 75-100% of the cell monolayer was affected. Cell cultures negative for CPE were frozen after 14 days of observation.

2. Second Passage

Cell cultures positive or negative for viral CPE on first passage routinely underwent a second passage. Cell cultures from the first passage were thawed once and the harvest fluids pooled. CPE positive and CPE negative cell cultures were pooled separately. Twenty percent

of each pooled harvest-fluid was passaged into additional cell cultures. Cell cultures negative for viral CPE on the 14th day of the 2nd passage were considered negative for virus. Cell cultures positive for CPE were confirmed for virus by an additional passage.

NOTE: To date, all the samples were assayed only in BS-C-1 cells. However, virus assays to be conducted during the next phase of this study will also be carried out in primary human embryonic kidney (HEK) cells.

F. IDENTIFICATION OF VIRAL ISOLATES

In some cases, a preliminary indication of the viral group was obtained from the characteristic CPE observed in infected cells and the length of time required for 100% CPE to occur. Electron microscopy was used to identify the viral group of the isolates. The viruses were typed by the neutralization test using specific antisera.

G. PROCEDURE FOR CONCENTRATING POLIOVIRUS FROM EXPERIMENTAL SAMPLES

An aliquot of stock poliovirus type 1, Sabin, was taken out of the freezer and thawed at room temperature. The virus was diluted in MEM E containing 2% FCS to the desired concentration and added to a conditioned sample of water (i.e., acidified water containing EBSS). The virus was concentrated by the membrane filtration method as described previously. Dilutions of the final concentrated eluate were prepared in media and inoculated into BS-C-1 cells. The cell cultures were observed for 7-10 days at which time the TCID₅₀ titres of the virus were calculated. A virus control was included in the test to determine the actual input virus. The titres of the input and recovered viruses were calculated and the efficiency of the method was expressed as the percentage of input virus recovered.

H. REGENERATION OF DUOFINE MEMBRANE FILTERS

It was found that the filters could be regenerated and reused up to 3 times before the water flow rates were reduced significantly. After use, the filter housings were filled with 800 mL of 0.1 N NaOH and left to stand at room temperature for a minimum of 2 hours. The NaOH solution was backflushed out of the housings with air pressure. This process was repeated with 0.01 N HCl and deionized water. Finally, the filters were decontaminated by autoclaving at 110°C for 15 min. and reused.

I. CONCENTRATION OF VIRUSES FROM SEWAGE BY THE GAUZE-PAD METHOD

1. Preparation of pads and sample collection

The gauze pads, measuring approximately 6" x 6", consisted of absorbent cotton bound with cheesecloth and tied with a 30 lb. fishing line. A No. 12 lead sinker was inserted into the middle of the pads for added weight and the pads were autoclaved at 121°C for 15 min. They

were suspended in the sewage flow for 48 hours. At the time of collection, excess fluid was squeezed out of the pads. They were placed in plastic bags, sealed and transported on ice packs to the laboratory where they were stored overnight at 4°C before processing.

2. Processing sewage pad samples

All of the residual fluid was squeezed out of the pad and poured into a sterile bottle labelled "O" for original fluid. The eluant, 100 mL of 0.05 M glycine, pH 11.5, containing 10% FCS was added to the plastic bag. The pad was soaked in the eluant for a few minutes before squeezing the fluid out of the pad again. The pH of the eluate was measured and adjusted with 1 N NaOH if necessary, to bring the pH up to 9.0-9.5.

The pad was resoaked and the procedure repeated 3-4 times. Approximately 100 mL of eluate was extracted from the pad and poured into a sterile bottle labeled "E" for eluate. A maximum of 100 mL of "O" fluid was mixed with 100 mL of the eluate. The pH was measured once again and raised to 9.0-9.5 if necessary. The mixture was centrifuged at 14 000 rpm for 30 min. at 5°C in a refrigerated centrifuge. Ten mL of the supernatant was removed and clarified by filtration through celite 503, an AP 25 prefilter and 0.45 µm membrane. The clarified supernatant was sterilized through a 0.45 µm membrane and neutralized with sterile 0.05 M glycine, pH 1.0 before being assayed for virus. The remainder of the original supernatant was stored at -20°C.

J. EXTRACTION OF VIRUSES FROM SEDIMENTS

1. Sample Collection

Sediment samples were collected in clean, 1 L, wide-mouthed, plastic bottles. The bottle was immersed in the water and run along the surface of the lake bed to collect the top 3 cm. of sediment. This process was continued until the container was 1/2 full. The sediment was allowed to settle for a few minutes before the excess water was poured off. The sediments were transported to the laboratory on ice and placed into storage at -20°C until processing.

2. Processing Sediment Samples

The frozen sediments were thawed and any residual water poured off. Fifty mL of eluting fluid, 0.05 M glycine, pH 11.5, containing 10% FCS, was added to the sample and mixed thoroughly by shaking. The sediments were allowed to settle and the pH of the supernatant was measured. If necessary, 1 N NaOH was added to raise the pH of the eluate to 9.0-9.5 and the sample was mixed again by shaking. The eluate was removed and clarified by filtration through celite 503, AP 25 prefilter and a 0.45 µm membrane. The clarified eluate was filter-sterilized and neutralized with sterile 0.05 M glycine, pH 1.0. Ten mL of the sample was assayed for virus and the remainder was stored at -20°C.

RESULTS

1. Concentration of Virus by Duofine Membrane Filtration

In order to determine the efficiency of recovering enteroviruses from large volumes of water by Duofine membrane filtration, 40 L volumes of lake or deionized water were experimentally contaminated with type 1 (Sabin) poliovirus. The virus was concentrated and assayed as described in Materials and Methods. The results are presented in Table 2.

Seventy percent of the input virus was recovered from lake water. The percent of virus recovered from deionized water ranged from 14 to 141.

2. Virological Analyses of Field Samples

Lake water and sediment samples from bathing beaches on the Great Lakes were collected for virological analysis (Table 3). The sites are those previously described in this report.

A total of 22 lake water and sediment samples were collected at the sampling sites listed in Table 3. Every 40 L sample of water was concentrated by Duofine membrane filtration and assayed as described in Material and Methods. The results are shown in Table 4.

All eleven water samples were found to be negative for virus after two consecutive passages of the sample-concentrates in BS-C-1 cells. The results were confirmed by a third cell culture passage. The sediment samples were negative after passages in BS-C-1 cells and were verified by a third passage.

3. Virological Analysis of Sewage and Receiving Water

Influent and effluent sewage samples and upstream and downstream receiving water samples were collected from a tertiary sewage treatment plant (STP), located in south-western Ontario. The STP discharges into a local river. At the time of sampling, the STP was participating in a study being conducted on the effect of chlorination on bacterial disinfection.

The first set of samples for virological was collected in December, 1979 when chlorination of the final effluent was shut off. The second set was collected in January, 1980 after the STP had resumed chlorination. Forty litre grab samples were collected from the sewage outfall and the upstream and downstream receiving waters. Sodium thiosulphate was added to the chlorinated and unchlorinated grab samples to ensure uniformity. Gauzed-pad samples were collected from influent and effluent sewages after 48 hrs. of immersion. All of the samples were processed as described in Materials and Methods and assayed for virus in BS-C-1 cell cultures. The results are given in Table 5.

During the period the STP was not chlorinating the final sewage effluent before discharging it into the river, all of the samples analysed were found to be positive for virus after 2 passages in cell cultures. Electron microscopic examination of the virus isolates revealed the presence of enteroviruses in the influent sewage (Figure 3) and enteroviruses and reoviruses in the effluent sewage. Only reoviruses (Figure 4) were isolated from the river samples. Each of the viral isolates will be typed by the neutralization test using specific antisera.

Viruses have also been isolated, after two cell culture passages, from all of the samples collected during the time that the STP was employing chlorination. Their identification is in progress.

Table 2 - Recovery of Poliovirus from 40 L Volumes of Water by Duofine Membrane Filtration

Sample	Experiment No.	Input Virus (TCID ₅₀ /mL)	Virus Recovered (TCID ₅₀ /mL)	% Virus Recovered
Deionized Water	1	1.26×10^4	1.78×10^4	141
	2	5.60×10^3	1.01×10^3	18
	3	3.20×10^2	1.70×10^2	53
	4	1.78×10^2	2.50×10^1	14
Lake Water	1	1.26×10^4	8.81×10^3	70

Table 3 - Collection of Water and Sediment Samples

Site	Location	Date Sampled
Jones Beach	Lake Ontario	Sept. 26, Oct. 10
Confederation Park	Lake Ontario	Sept. 26
Hanlans Point Beach	Lake Ontario	Oct. 1
Breakwater Beach	Lake Ontario	Oct. 1
Lakeside Park	Lake Ontario	Oct. 10
Wasaga Beach No. 5	Georgian Bay, Lake Huron	Oct. 16
Wasaga Beach No. 2	Georgian Bay, Lake Huron	Oct. 16
Ipferwash Beach	Lake Huron	Oct. 21
Grand Bend Beach	Lake Huron	Oct. 21
Iroquois Beach Provincial Park	Lake Erie	Oct. 24

Table 4 - Virological Analyses of Lake Water and Sediment Samples

Type of Sample	No. Analysed	No. Positive
Lake Water	11	0
Sediments	11	0

Table 5 - Virological Analyses of Sewage and Receiving River Water Samples

	Chlorination Off ^(a)		Chlorination On ^(b)	
	Cell Cultures ^(c)	EM	Cell Cultures	EM
Influent Sewage (gauze pad)	+	Enterovirus	+	ND ^(d)
Effluent Sewage 1. (gauze pad) 2. (40 L grab)	+	Reovirus	+	ND
	+	Enterovirus	+	ND
Receiving River Water Downstream (40 L grab)	+	Reovirus	+	ND
Receiving River Water Upstream (40 L grab)	+	Reovirus	+	ND

(a) STP was not employing chlorination

(b) STP was employing chlorination

(c) Presence or absence of Viral CPE

(d) Not done

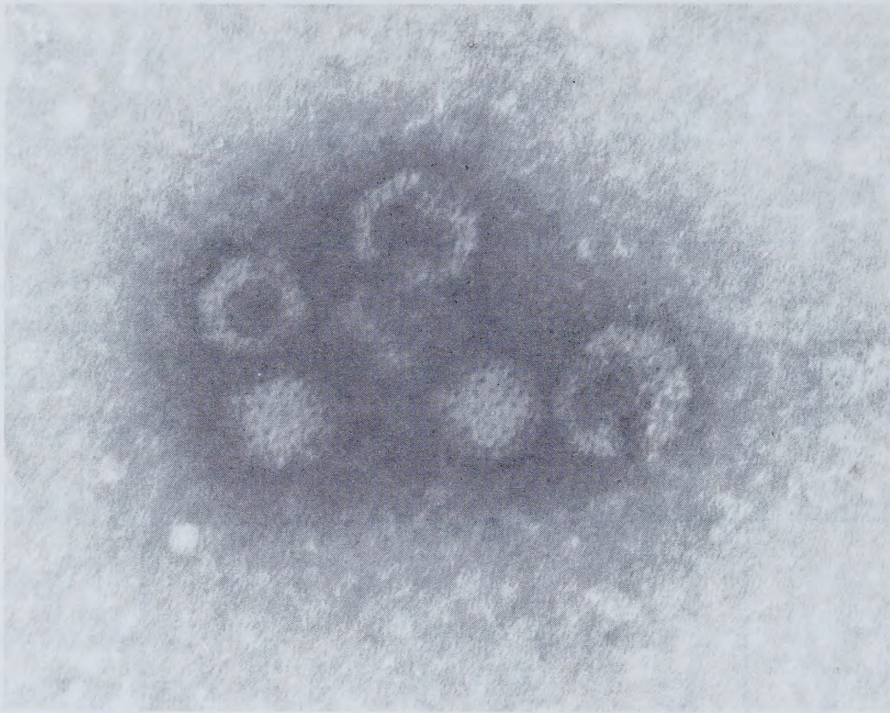


Figure 3 - Electron micrograph of a cluster of negatively stained enteroviruses
Magnification x 200 000

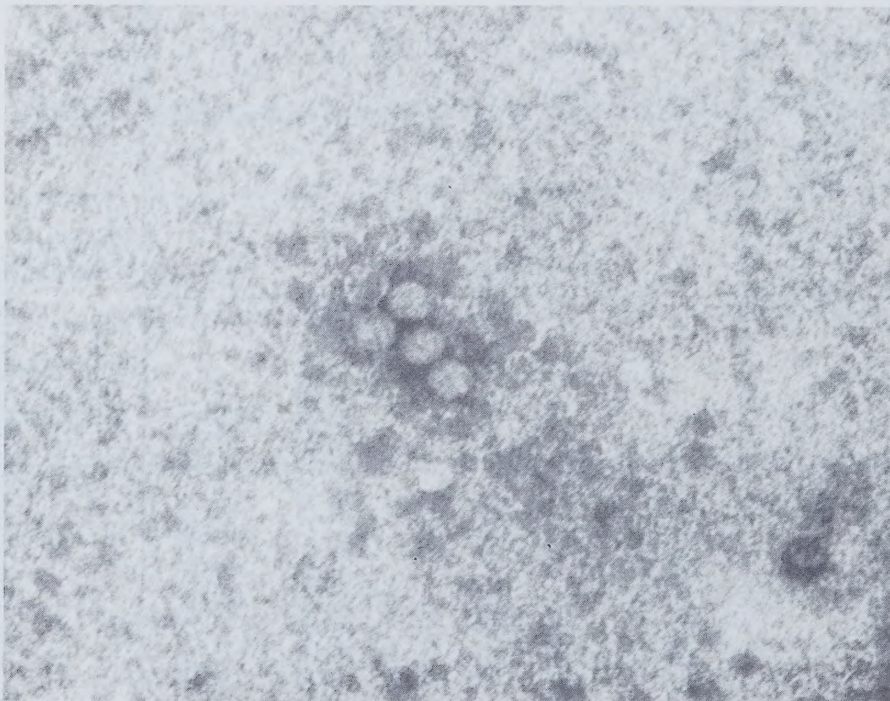


Figure 4 - Electron micrograph of a cluster of negatively stained reoviruses
Magnification x 200 000

DISCUSSION

The Duofine membrane filtration method for concentrating viruses from water and waste-water was found to be practical and relatively inexpensive. It could be adapted to a wide variety of waters with appropriate modifications. A preliminary evaluation of the efficiency of the method for concentrating poliovirus from experimentally contaminated water was conducted. The rate of virus recovery ranged from 14 to 141%. These experiments were not performed under the most ideal conditions and therefore, further experiments are needed for an accurate assessment of the efficiency of this method.

The isolation of viruses from sewage and receiving river water demonstrated that these methods are capable of detecting the presence of enteroviruses and reoviruses in the environment.

The isolation of viruses from both chlorinated and unchlorinated sewage effluents from a tertiary sewage treatment plant, emphasizes the shortcomings of current chlorination practices. Consequently, viruses are discharged into the receiving waters where they pose a potential health hazard.

Samples from the bathing beaches were collected in late September and October when there were no swimmers present. No viruses were isolated from any of the bathing beach sites examined. This does not necessarily mean that these waters were free of virus, but it does indicate that they were below the detection limits of these methods.

SUMMARY

1. Preliminary evaluation of the Duofine Membrane filtration method revealed virus recovery rates ranging from 14 to 141 percent.
2. Virological analyses of lake water and sediment samples revealed no evidence of virus.
3. Examination of influent and effluent sewages and upstream and downstream receiving waters resulted in the isolation of enteroviruses and reoviruses.

REFERENCES

1. Melnick, J.L., C.P. Gerba, and C. Wallis. 1978. Viruses in Water. Bull. WHO, 56: 499-508.
2. Rhodes, A.J., E.M. Clark, D.S. Knowles, et al. 1950. Poliomyelitis Virus in Urban Sewage. Can. J. Publ. Hlth., 41: 248.
3. Nestor, I., and L. Costin. 1976. Presence of certain enteroviruses (coxsackie) in sewage effluents and in river waters of Roumania. J. Hyg. Epidemiol. and Immunol., 20: 137-149.
4. Payment, P., Y. Larose and M. Trudel. 1979. Polioviruses and other enteroviruses in urban sewage from Laval (Canada): presence of non-vaccinal strains of poliovirus. Can. J. Microbiol., 25: 1305-1309.
5. Berg, G. (ed.). 1967. Transmission of viruses by the water route. John Wiley and Sons, New York.
6. Grinstein, S., J.L. Melnick, and C. Wallis. 1970. Virus isolations from sewage and from a stream receiving effluents of sewage treatment plants. Bull. WHO, 42: 291-296.
7. Lamb, G.A., T.D.Y. Chin, and L.E. Scarce. 1964. Isolations of enteric viruses from sewage and river water in a metropolitan area. Am. J. Hyg., 80: 320-327.
8. Hawley, H.B., D.P. Morin, M.E. Geraghty, J. Tomkow, and C.A. Phillips. 1973. Coxsackie virus B epidemic at a boy's summer camp: Isolation of virus from swimming water. J. Am. Med. Assoc., 226: 33-36.
9. Melnick, J.L. 1978. Water as a reservoir of virus in nature and means for control. In: Kurstak, E. and Maramorosch, K. eds. Viruses and Environment, pp. 203-226. Academic Press, New York.
10. Mosley, J.W. 1967. Transmission of viral diseases by drinking water. In: Berg, G. ed. Transmission of viruses by the water route, pp. 5-23. Inter-Science Publishers, New York.
11. Plotkin, S.A., and M. Katz. 1967. Minimal infective doses of viruses for man by the oral route. In: Berg, G. ed. Transmission of viruses by the water route, pp. 151-166. John Wiley and Sons, New York.
12. MacLean, D.M. 1973. Detection and inactivation of enteroviruses in water. National Research Council Canada, Publication No. BY72-4 (ES).
13. Sattar, S.A. 1978. Viruses, Water and Health. University of Ottawa Press, Ottawa.

14. Gerba, C.P., S.R. Farrah, S.M. Goyal, C. Wallis, and J.L. Melnick. 1978. Concentration of enteroviruses from large volumes of tap water, treated sewage, and seawater. *Appl. Environ. Microbiol.*, 35: 540-548.
15. Sobsey, M.D., and B.L. Jones. 1979. Concentration of poliovirus from tap water using positively charged microporous filters. *Appl. Environ. Microbiol.* 37: 588-595.
16. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1976. Detection of enteric viruses in water and wastewater. In: *Standard methods for the examination of water and wastewater*, pp. 968-975, 14th ed. American Public Health Association, Washington, D.C.
17. Environmental Research Center. 1978. Human viruses in the aquatic environment: A status report with emphasis on the EPA research program. Report to Congress.

APPENDIX C

DESCRIPTION OF BEACH SAMPLING SITES

Table of Contents

	<u>Page</u>
<u>LAKE ERIE</u>	
HOLIDAY BEACH	111
IROQUOIS BEACH	111
LONG POINT	111
POINT PELEE	111
PORT BRUCE	112
PORT DOVER	112
PORT STANLEY	112
RONDEAU PARK	112
SELKIRK	113
TURKEY POINT	113
 <u>LAKE HURON</u>	
GRAND BEND	113
IPPERWASH	114
KILLBEAR POINT	114
PINERY PARK	114
WASAGA BEACH	114
 <u>LAKE ONTARIO</u>	
DARLINGTON PARK	115
HAMILTON PARK	116
CHARLES DALEY PARK	116
CONFEDERATION PARK	116
JONES BEACH	116
LAKESIDE COURT	117
LAKESIDE PARK	117
MUNICIPAL BEACH (WELLER PARK)	117
VAN WAGNERS BEACH	117
HANLANS POINT	118
BREAKWATER BEACH	118
OLYMPIC BEACH	119

HOLIDAY BEACH PROVINCIAL PARK LAKE ERIE

Holiday Beach Provincial Park is located at the western end of Lake Erie, about 40 kilometres out of Windsor. It is a recreational park with limited camping facilities. Facilities include: parking, campsites, washrooms, change rooms, picnic tables, playgrounds, boat launching facilities and a stocked fish pond. The beach, which is bordered by trees, shrubs and tall grass, is long wide and flat. A river empties into Lake Erie, nearby. Both the beach and lake bottom, are covered with fine, consistent sand. The beach sand is dry and the water is shallow. The sampling site itself, was east of a pier.

IROQUOIS BEACH PROVINCIAL PARK LAKE ERIE

Iroquois Beach Provincial Park is located on Lake Erie, west of the commercial fishing harbour at Port Burwell. A river empties into Lake Erie, via Port Burwell harbour, nearby. It is a recreational park with camping facilities. These facilities include: parking, campsites, picnic tables, washrooms, change rooms, playgrounds, playing fields, boat launching facilities and nearby boat rental services. The beach, which is bordered by small trees, shrubs and dune grass, is long, wide and flat. The beach sand is fine, consistent and dry. The water is shallow and the lake bottom is covered with fine, consistent sand.

LONG POINT PROVINCIAL PARK LAKE ERIE

Long Point Provincial Park is located on a peninsula, towards the eastern end of Lake Erie. It is a recreational park with camping facilities. Many private cottages are found in and around the provincial park. Facilities include: parking, campsites, picnic tables, washrooms, change rooms, boat launching facilities and nearby boat rental services. The beach, which is bordered by sand dunes, is long, wide and flat. Both the beach and lake bottom are covered with fine, consistent sand. The beach sand is dry and the water is shallow.

POINT PELEE NATIONAL PARK LAKE ERIE

Point Pelee National Park is located on a large peninsula, at the western end of Lake Erie. The park has two major beaches, one on each side of the peninsula. Our sampling site was on the Northwest Beach. The park's facilities include: parking, campsites, washrooms, change

rooms, picnic tables, playgrounds and boat launching facilities. The beach, which is bordered by large bushes, shrubs and a few trees, is long, wide and flat. Both the beach and lake bottom are covered with fine, consistent sand. The beach sand is dry and the water is shallow.

PORT BRUCE PROVINCIAL PARK LAKE ERIE

Port Bruce Provincial Park, which is located in the village of Port Bruce, is classified as a recreational park with day use facilities only. A river empties into Lake Erie, via Port Bruce Harbour, nearby. Facilities include: washrooms, change rooms, picnic tables and nearby boat docking facilities. The beach, which is bordered by cottages and a few trees, is short, wide and flat. The sand is fine, consistent and dry. The water is shallow and the sandy lake bottom is fine and consistent.

PORT DOVER BEACH LAKE ERIE

Port Dover Beach is a small municipal beach, located on Lake Erie, east of Port Dover harbour. A river empties into Lake Erie, via Port Dover harbour, nearby. The beach, which has no facilities itself, is surrounded by commercial development. There are a few trees growing on the short and narrow beach. Both the beach and lake bottom are covered with granular, consistent sand. The sand is dry and the water is quite shallow.

PORT STANLEY BEACH LAKE ERIE

Port Stanley Beach is a small municipal beach, located on Lake Erie, east of Port Stanley harbour. A river empties into Lake Erie, via Port Stanley harbour, nearby. There are no facilities. The beach is short, narrow and flat, and it is bordered by homes, cottages and commercial development. Both the beach and lake bottom are covered with fine, consistent sand. The beach sand is dry and the water is relatively shallow.

RONDEAU PROVINCIAL PARK LAKE ERIE

Rondeau Provincial Park, which is located on a peninsula in Lake Erie, is classified as a natural environment park with camping facilities. It is a major Ontario reserve for birds and

Carolinean forest (tulip trees, walnut and sassafras). The park is facilitated with: parking, campsites, picnic tables, washrooms, change rooms, boat launching facilities and nearby boat rental services. The beach, which is bordered by forest, is long, wide and flat. Both the beach and lake bottom are covered with fine, consistent sand. The beach sand is dry and the water is shallow.

SELKIRK PROVINCIAL PARK LAKE ERIE

Selkirk Provincial Park, which is located at the eastern end of Lake Erie, is a recreational park with camping facilities. A river flows into Lake Erie, west of the park. Facilities include: parking, campsites, picnic tables, washrooms, change rooms and boat launching facilities. The beach area sampled was short, wide, flat and bordered by trees. The beach sand and lake bottom are granular and consistent. The beach sand is dry and the water is quite shallow.

TURKEY POINT PROVINCIAL PARK LAKE ERIE

Turkey Point Provincial Park, which is located on Long Point Bay, is classified as a natural environment park with camping facilities. These facilities include: parking, campsites, picnic tables, playgrounds, golf, nature trails and nearby boat rental services. The park is bordered by homes and cottages. The beach itself, is long, wide, flat and bordered by trees. The beach and lake bottom are covered with fine, consistent sand. The beach sand is dry and the water is shallow.

GRAND BEND BEACH LAKE HURON

Grand Bend, situated on Lake Huron, 43 miles from London, Ontario, has a normal population of 800 residents. During the height of the summer, the population swells to three or four thousand. A sewage system, currently being installed in the town, will not be operational until the summer of 1981. The Ausable River flows into the lake immediately south of the beach area. Grand Bend has a wide expanse of beach with virtually no vegetation on it. There is a bath house located in the center of the beach, close to the main street. The soil type found throughout the Grand Bend area is sand and the beach consists of medium sized sand particles. The nearshore lake bottom is usually stony. There are sandbars in the lake, but the location of these will vary from year to year.

IPPERWASH BEACH

LAKE HURON

Ipperwash is an area of large sand dunes, which gradually level off close to the lake. The Ipperwash beach is used by the public, by campers at the Provincial Park and by young people at the Cadet Camp. Vegetation close to the lake is sparse, but there are heavily wooded areas inland. The beach surface and the lake bottom, both consist of medium sized sand particles. The water level in the lake drops off gradually, and sediment sampling is relatively easy.

KILLBEAR POINT PROVINCIAL PARK (Georgian Bay)

LAKE HURON

Killbear Point is located in the typically rocky and wooded Georgian Bay area, near Parry Sound. The park is essentially designed for camping and boating and it is equipped with extensive washroom facilities and boat docks. There are two small beach sites in the park that are designated as swimming areas. The water is shallow at these locations and both the beach surface and the lake bottom are sandy.

PINERY PROVINCIAL PARK

LAKE HURON

The entire park is composed of a series of sand dunes ranging from ninety feet high near the park entrance to only a few feet high at the beach. Most of the area is heavily wooded with the Ausable River flowing through the center and out into the lake. The park is divided into a camping section and a section for day use. There are nine separate beach areas with parking facilities at each. The sandy beach is similar to Ipperwash and the lake bottom is composed of sand.

WASAGA BEACH PROVINCIAL PARK (Georgian Bay)

LAKE HURON

Wasaga Beach Provincial Park consists of nine miles of continuous beach on Nottawasaga Bay. The park is subdivided into eight areas. Our studies concentrated on three of these areas: Beach Area 5, Beach Area 2, and New Wasaga Beach Area. The Provincial Park has many facilities: picnic tables, playgrounds, game courts, change rooms, washrooms, activity centres, first aid centres, parking, boat launching facilities and a forum amphitheatre. Although Wasaga Beach Provincial Park is classed as a recreational park having day use facilities only, overnight accommodations are provided by private campgrounds, motels and cottages in and around the park.

NEW WASAGA BEACH AREA

New Wasaga Beach Area is bordered by private cottages and some commercial development. At the south end of this beach area, the Nottawasaga River empties into the lake. Dune grasses and shrubs grow along the periphery of the beach and a small stream empties into the lake near the sampling site. The beach itself is wide and flat. The beach sand is fine, smooth, consistent and damp, which results in its tendency to pack down. The sandy lake bottom is also fine, smooth and consistent. The water is shallow and there are numerous sandbars.

WASAGA BEACH AREA 2

Beach Area 2 is bordered by parkland, consisting of cut grass and trees, with many of the facilities previously mentioned. The north end of this beach area is commercially well developed with stores, restaurants, snack bars and amusement areas. A boardwalk runs along the periphery of the wide flat beach. The beach sand is smooth, consistent and dry. The sandy lake bottom is also fine, smooth and consistent. The water is shallow and there are numerous sandbars.

WASAGA BEACH AREA 5

Beach Area 5 is mainly bordered by parkland, although private cottages and stores are to be found on the beach. Except for the absence of a boardwalk, the parkland facilities are similar to those of Beach Area 2. The beach itself is relatively narrow when compared with the other two sampling sites. The beach sand is granular, consistent, and packs down when damp. Like the rest of the provincial park, the sandy lake bottom is fine, smooth and consistent, the water is shallow and the sandbars are numerous.

DARLINGTON PROVINCIAL PARK LAKE ONTARIO

Darlington is a large park with an extensive picnic area and facilities for boating. There is a sizeable trailer camp in the eastern portion of the park. The terrain is rolling and for the most part covered by cut grass. The park is lightly treed with the exception of the trailer park which is wooded. The bathing beach is approximately 20 metres wide, and is covered with coarse grained sand. The lake bottom in the swimming area tends to be stony. Although Darlington Park is a relatively short distance east of Toronto, swimming activities at the site are limited because of the generally cold temperatures of the water.

HAMILTON PARK
LAKE ONTARIO

Hamilton Park is physically located in Burlington, opposite the Canada Centre for Inland Waters building. The park does not have much aesthetic appeal because although there is a wide stretch of sandy beach, the area is littered with garbage and there is a train track through the centre. On the positive side, there is a bath house at the site and the lake bottom is sandy. The beach is apparently crowded during very hot weekends in the summer.

CHARLES DALEY PARK
LAKE ONTARIO

Charles Daley Park, which is owned, operated and maintained by the Niagara Parks Commission, is located on Lake Ontario, west of the City of St. Catharines. It is a recreational park with overnight camping facilities. The facilities include: campsites, picnic areas, washrooms, change rooms, playgrounds, a snack bar, a grocery store and nearby boat launching facilities. The park is bordered by highway and farmland. Two creeks empty into Lake Ontario within the park. The beach, which is bordered by cut grass, is covered with stones. The gently sloping lake bottom is sandy, granular and consistent, with an accumulation of stones near shore.

CONFEDERATION PARK
LAKE ONTARIO

Confederation Park is a municipal park located on Lake Ontario, in the City of Hamilton. It is a recreational park with overnight camping facilities. These facilities include: parking, campsites, picnic areas, washrooms, change rooms, snack bars, playground equipment and nearby boat launching facilities. The long, wide, sandy beach, which has a gentle slope, is bordered by lawns and trees. The sand is granular, consistent and dry. The lake bottom has a gentle slope and is covered with granular, consistent sand.

JONES BEACH
LAKE ONTARIO

Jones Beach is a small municipal park located east of Port Weller Harbour in the City of St. Catharines. The Welland Ship Canal empties into Lake Ontario, via Port Weller Harbour, nearby. The park is bordered by the Welland Ship Canal and industrial development. There are no facilities. The beach, which is bordered by trees, tall grass and a service road, is wide, flat and rocky. The edge of the beach and lake bottom is usually covered with a thick layer of algae and decaying organic matter. The water is shallow.

LAKESIDE COURT
LAKE ONTARIO

Lakeside Court (not to be confused with Lakeside Park) is located on Lake Ontario near Beamsville. The court is bordered by a marina and a few cottages. The beach itself, is short, narrow and rocky. The lake bottom is rocky near shore, but further out, it is sandy and consistent. Algae usually grows near the shore. The slope of the lake bottom is steep.

LAKESIDE PARK
LAKE ONTARIO

Lakeside Park (not to be confused with Lakeside Court) is a small municipal park located on Lake Ontario, just west of Port Dalhousie Harbour in the City of St. Catharines. It is a recreational park with day use facilities only. The park is bordered by residential and commercial development. Martindale Pond empties into Lake Ontario, via Port Dalhousie Harbour, at the eastern end of the park. Facilities include: parking, washrooms, change rooms, a covered picnic area, a snack bar and nearby boat docking facilities. The wide, flat beach is bordered by cut grass and trees, and the beach sand is granular, consistent and dry. The water is shallow and the sandy lake bottom is fine, smooth and consistent.

MUNICIPAL BEACH (WELLER PARK)
LAKE ONTARIO

Municipal Beach (Weller Park, City Park of St. Catharines) is a municipal park located west of Port Weller Harbour in the City of St. Catharines. It is a recreational park with day use facilities only. The Welland Ship Canal empties into Lake Ontario, via Port Weller Harbour, nearby. The park is surrounded by residential areas and municipal facilities. The park has parking, picnic tables, washrooms, change rooms, playground equipment, a snack bar and a boat launching area. The wide, flat beach is bordered by cut grass and trees. The beach sand is granular, consistent and dry. The water is shallow and the sandy lake bottom is granular and consistent with small stones near the shore.

VAN WAGNERS BEACH
LAKE ONTARIO

Van Wagners Beach is located on Lake Ontario, just northwest of Confederation Park in the City of Hamilton. It is bordered by highway. The beach itself, has no facilities, but a

snack bar and boat launching facilities are shared with Confederation Park. A fence separates the beach from the road nearby. The beach is long, wide and flat with a few trees. The sand is granular, consistent and dry. The lake bottom has a gentle slope and is covered with granular, consistent sand.

TORONTO ISLAND PARK BEACHES LAKE ONTARIO

Toronto Island Park, under the jurisdiction of the Metropolitan Toronto Parks Department, is a recreational park with day use facilities only. It is well supplied with picnic tables, washrooms, change rooms, wading pools, snack bars and first aid centres. There are restaurants, boat docking facilities and a children's amusement area. A residential community is to be found on Ward's and Algonquin Islands. Access to the park, is provided by ferry boats between the city and three docks on the island. Of the five beaches on the island, only three were sampled: Hanlans Point Beach, Breakwater Beach and Olympic Beach.

HANLANS POINT BEACH

Hanlans Point Beach is on the Lake Ontario side of the island, facing west. It is the longest of the three beaches sampled. The northern half of the beach is bordered by the Island Airport, while the southern half is bordered by trees, shrubs, and tall grass. The width of the beach varies, but it tends to narrow at the center. The slope is gentle and the beach sand is granular, consistent and dry. The sandy lake bottom is granular with many stones, except for a small area at the southern end of the beach which is rocky.

BREAKWATER BEACH

Breakwater Beach is located on the Lake Ontario side of the island, facing south. A service road separates the beach from the parkland, which consists of cut grass and trees. Washrooms, change rooms, a snack bar and pier are to be found at the eastern end. Playground equipment, a few trees and grass are located on the beach itself. The beach is wide, flat and covered with dry, granular, consistent sand. The sandy lake bottom is fine, smooth and consistent. The water is shallow and protected along its entire length by a breakwater made of heavy boulders.

OLYMPIC BEACH

Olympic Beach, which is the smallest and narrowest of the three beaches sampled, is located on the Toronto Harbour side of the islands, facing north. It is bordered by cut grass and trees. A complex, containing washrooms, change rooms, a snack bar and first aid centre is located on the beach's edge. Playground apparatus has been built on the beach itself. The sand is granular, consistent and dry. The lake bottom is mainly fine, smooth, consistent sand, with an accumulation of stones by the edge of the water. The water is shallow. Because of the high bird population, there is a concentration of organic matter in this area.

APPENDIX D

MICROBIOLOGICAL METHODS

Table of Contents

	<u>Page</u>
A. COLLECTION OF SAMPLES.....	125
B. SAMPLING LOCATION	125
Surface Water Samples	125
Sediment Samples	125
C. PREPARATION OF SEDIMENT FOR ANALYSIS.....	125
Method of Gerba <u>et al</u> , 1979	125
Method of Seyfried, Vidgen, Bednarski and Nishikawa unpublished.....	126
D. METHOD FOR DETERMINATION OF VIABLE HETEROTROPHIC BACTERIAL COUNTS (YOUNG, 1978)	126
E. PROCEDURE FOR IDENTIFICATION OF HETEROTROPHIC ORGANISMS.....	127
F. PRELIMINARY BIOCHEMICAL TESTS USED FOR HETEROTROPHIC DETERMINATION	128
G. METHOD FOR DETERMINATION OF FECAL COLIFORMS	131
MOST PROBABLE NUMBER TECHNIQUE FOR FECAL COLIFORMS (APHA, 1975).....	131
MEMBRANE FILTRATION METHOD FOR FECAL COLIFORMS (DUTKA, 1978)	132
MEMBRANE FILTER TECHNIQUE FOR FECAL COLIFORMS (PAGEL and VLASSOFF, 1979).....	133
H. METHOD FOR DETERMINATION OF FECAL STREPTOCOCCI	133
MEMBRANE FILTER TECHNIQUE FOR FECAL STREPTOCOCCI (APHA, 1971).....	133
MEMBRANE FILTER TECHNIQUE FOR FECAL STREPTOCOCCI (APHA, 1975).....	134
I. METHOD FOR DETERMINATION OF STAPHYLOCOCCUS AUREUS.....	135
MEMBRANE FILTRATION METHOD FOR ISOLATION OF Staphylococcus aureus	135
ADDITIONAL STAPHYLOCOCCAL TESTS	136
J. METHOD FOR DETERMINATION OF Pseudomonas aeruginosa	137
MOST PROBABLE NUMBER TECHNIQUE FOR Pseudomonas aeruginosa (APHA, 1975).....	137
MEMBRANE FILTRATION TECHNIQUE FOR Pseudomonas aeruginosa (APHA, 1975).....	139
REFERENCES	141

MICROBIOLOGICAL METHODS

A. COLLECTION OF SAMPLES

Bacteriological samples should be collected in sterilized containers. The samples should be chilled on ice (not frozen) and transported to the laboratory, where analysis must commence within 6 hours of the sampling time.

It is the responsibility of the sampler to use aseptic techniques when handling sterile containers.

B. SAMPLING LOCATION

Select a location with a high density of swimmers. Walk directly away from shore until the water depth reaches approximately 50 cm, and collect both the surface water and sediment samples from that site.

Surface Water Samples

Touch only the outer surface of the cap when opening the bottle. The recommended procedure is to immerse the closed bottle under the surface of the water and to then remove the cap, holding the cap with one's fingertips under the water until the sample has been taken. The sample bottle is held below the surface of the water until air bubbles have stopped coming to the surface. When sampling near shore, care should be taken to get a sample uncontaminated with sediment.

Sediment Samples

Sediment samples are collected in clean, dry, wide-mouthed, plastic or metal containers about 500 mL in size. The container is immersed in the water and run along the surface of the lake bed, to collect the top 3 cm of sediment. This process is continued until the container is at least 1/2 full of sediment. The sediment containers are chilled (not frozen) and transported to the laboratory in the same manner as the surface water sample bottles.

C. PREPARATION OF SEDIMENT FOR ANALYSIS

Method of Gerba et al. (1979)

Sediment samples were initially diluted 1:1 by mixing 170 gm (wet weight) of sediment with 170 mL of cold, sterile, phosphate buffered water. The mixture was then blended at low speed for 1 minute. The sediment was allowed to settle before using the supernatant. This supernatant contained approximately 0.5 gm of sediment per mL.

Method of Seyfried, Vidgen, Bednarski and Nishikawa, unpublished

Mix wet sediment samples thoroughly and pack a portion of the mixed sample into a tared (pre-weighed) graduated cylinder to a volume of 50 mL (approximately 100 gm). Weigh the cylinder containing the wet sediment to the nearest mg. Calculate the wet weight of the sediment by subtracting the initial weight of the cylinder from the final weight of the cylinder and wet sediment.

Prepare sediment dilutions by adding 50 mL phosphate buffered water to the 50 mL of wet sediment in the cylinder. Blend the sediment suspension for 1 minute using a mechanical blender at low speed. Turn off the blender and let stand a few minutes to facilitate the settling of the sediment. Carefully decant the supernate into a suitable size flask. Thoroughly mix the supernate immediately before further analysis of dilutions are performed.

D. METHOD FOR DETERMINATION OF VIABLE HETEROTROPHIC BACTERIAL COUNTS
(YOUNG, 1978)

Procedure for Surface Water Samples

The required, pre-dried Heart Infusion Agar (HI) and Casein-Peptone-Starch (CPS) dishes are set up and labelled. The range of dilutions selected for plating is primarily a subjective decision and is based on the appearance of the water sample. If the sample is relatively clean, smaller dilutions are used; however, if the sample is murky or dirty-looking, higher dilutions are used.

Aseptic technique is observed throughout this procedure. Assuming the sample is fairly clean the following procedure is utilized.

The flask containing the water sample is vigorously shaken.

A one mL aliquot of the water sample is withdrawn by sterilizing the flask opening using a bunsen burner. The one mL aliquot is delivered into a 9.0 mL sterile, buffered water tube. This constitutes a 10^{-1} dilution.

The 10^{-1} dilution tube is vortexed to obtain an even distribution of the inoculum, and then another one mL aliquot is aseptically withdrawn and transferred to 9.0 mL of sterile, buffered water. This constitutes a 10^{-2} dilution. This 10^{-2} dilution tube is again vortexed to obtain an even distribution. This same procedure is followed for each desired dilution.

Next, using one pipette and beginning with the greatest dilution (to minimize error), 0.1 mL aliquots of each dilution are placed onto appropriately labelled HI and CPS plates. After delivering the 0.1 mL aliquots to the agar surface, each is spread evenly over the surface of the agar with a sterile Drygalski bent glass rod.

The HI agar plates are incubated at 37°C for 48 hours.

The CPS agar plates are incubated at 20°C for 7 days.

The number of colonies on each plate is then counted after the appropriate periods of incubation, using a hand tally counter and stereoscopic microscope or magnifier at 10x magnification.

Procedure for Sediment Samples

The procedure employed for processing sediment samples to determine heterotrophic bacterial counts, is very similar to that of surface water, discussed above. However, due to the greater density and consistency of the sediment and hence greater number of bacteria, in comparison to lake water, much higher dilutions are used when processing sediment.

The HI and CPS agar Petri plates are appropriately labelled according to the desired dilutions, and septic technique is again imperative throughout this method.

The sediment supernatant is prepared by blending the sediment sample with sterile buffered water in a 1:1 ration, in a Waring blender for 1 minute (see Preparation of Sediment for Analysis Section). One mL of this supernatant is transferred aseptically into 9.0 mL of sterile, buffered water. This constitutes a 10^{-1} dilution. This dilution tube is vortexed and then one mL is aseptically withdrawn and pipetted into 9.0 mLs of sterile, buffered water. This constitutes a 10^{-2} dilution. The 10^{-2} diluted tube is then vortexed. This same procedure is continued until the desired dilutions are obtained.

The remainder of the procedure is exactly the same as that for surface water. 0.1 mL aliquots of each dilution are deposited on the surface of each agar (HI and CPS) and spread evenly with a Drygalski bent glass rod.

The HI plates are incubated at 37°C for 48 hours.

The CPS agar plates are incubated at 20°C for 7 days.

The number of colonies on each plate is then counted after the appropriate periods of incubation, using a hand tally counter and a stereoscopic microscope or magnifier at 10x magnification. Results are multiplied by two to account for the dilution factor.

E. PROCEDURE FOR IDENTIFICATION OF HETEROTROPHIC ORGANISMS

Total Plate Count of Heterotrophic Bacteria

Plate counts of the heterotrophic population present in surface water and sediment were made using Heart Infusion Agar and Casein-Peptone-Starch Agar.

Characteristic Determinations

Numerous identification schemes were examined in an effort to select the most efficient and practical method of determining the characteristics of lake organisms. The scheme proposed by Cowan and Steel (1974) uses reliable procedures and includes all of the organisms which predominate in water. Their identification charts for Gram-positive and Gram-negative bacteria are shown in Tables 1B and 2B, on the following pages.

Isolation and Purification of Cultures

The total plate count plates which contained approximately 50 colonies were used for isolation of cultures. The plates were selected to obtain a representative sample of the total bacterial population present. All the colonies were picked and streaked on Trypticase Soy Agar (BBL) plates and incubated at 35°C for 24 hours or until viable, visible colonies were obtained. Trypticase Soy Agar slants were prepared from the plates containing well-isolated colonies for use in further diagnostic testing procedures.

F. PRELIMINARY BIOCHEMICAL TESTS USED FOR HETEROTROPHIC DETERMINATION

Cellular Morphology and Gram Reaction

Hucker's modification of the Gram stain was used to study the microscopic appearance of organisms from 24-hour cultures grown on TSA. Cell shape was described using the criteria, length:width ratio greater than 2:1 rods, 1-2:1 coccobacilli, and 1:1 cocci. Where several shapes were present in one preparation, the one which seemed most prevalent was described as characteristic.

Spore stains were carried out on Gram-positive bacteria using the Schaeffer and Fulton malachite green method.

Colonial Morphology

Well isolated colonies on TSA plates were observed for colonial morphology after 48 hours of growth. Colour, consistency, and diffusible, brown pigment production were included in the analysis.

Motility Studies

A twice-transferred TSB culture was used to inoculate 10 mL of Motility Test Medium (Difco). The medium within the central Craigie tube was stab-inoculated to a depth of approximately 5 mm. Observation of motility after 48 hours and 1 week was facilitated by the incorporation of 0.005% triphenyl tetrazolium chloride into the medium. Where results were doubtful, motility was retested using the hanging drop method with two passages through TSB.

Table 1B - First-stage table for Gram-positive bacteria

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Shape	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Acid fast	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	w	+
Spores	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—
Motility	—	—	—	—	+	—	—	+	—	—	+	—	—	—	—	—	D	D	—	—	—
Growth in air	+	+	+	+	+	+	—	+	+	+	+	+	+	—	—	—	—	+	+	+	+
Growth anaerobically	—	+	w	w	+	+	+	—	+	+	+	+	—	+	+	+	+	D	—	—	x
Catalase	+	+	w	—	—	—	—	+	+	+	+	—	+	+	—	—	—	+	+	+	+
Oxidase	—	—	—	—	—	—	—	—	—	—	—	—	x	x	x	x	x	d	—	—	—
Glucose (acid)	D	+	+	+	+	+	+/-	—	—	+	+	+	+	+	+	—	D	D	+	+	+
OF	O/-	F	F	F	F	F	F/-	—	—	F	F	F	F	F	F	—	F/-	F/O/-	O	O	O/NT

<i>Micrococcus</i>	+	6.2																			
<i>Staphylococcus</i>		+																			
<i>Aerococcus</i>			+	+																	
<i>Streptococcus</i>					+	+															
<i>Pediococcus</i>						+															
<i>Gemella</i>						+															
Anaerobic cocci*							+														
<i>Kurthia</i>								+													
<i>Corynebacterium</i>									+	+											
<i>Listeria</i>										6.5	+										
<i>Erysipelothrix</i>												+									
<i>Lactobacillus</i>													6.6	+							
<i>Arachnia</i> †														+							
<i>Rothia</i>															+						
<i>Propionibacterium</i>																+					
<i>Actinomyces</i>																	+				
<i>Bifidobacterium</i>																		+			
<i>Eubacterium</i>																			+	6.8	
<i>Clostridium</i>																				+	
<i>Bacillus</i>																					6.9
<i>Nocardia</i>																				+	+
<i>Mycobacterium</i>																					6.10

* *Peptococcus*, *Peptostreptococcus* (also *Leuconostoc*).

† Also *Actinomyces odontolyticus*.

D Different reactions in different species of the genus.

d Different reactions in different strains.

F Fermentation.

O Oxidation.

w Weak reaction.

x Not known.

<> Asporogenous variants.

Typical form

Cultural characters of these organisms can be found in tables with the number indicated.

S Sphere (coccus).

R Rod-shaped (bacillus).

NT Not testable.

Table 2B - First-stage table for Gram-negative bacteria

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Shape	R	S	S	S	S/R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	-	-	-	-	-	-	+	+	+	+	+	+	+	D	-	-	+	-
Growth in air	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-†	+
Growth anaerobically	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+
Catalase	d	D	+	+	+	+	+	+	+	+	+	+	+	D	-	D	-	-
Oxidase	-	x	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-
Glucose (acid)	D	-	+	-	+	-	+	-	+	+	+	+	+	D	-	-	-	+
Carbohydrates [F/O/-]	F/-	-	O	-	O	-	O	-	O	O	F	F	F	F	NT	-	-	F

<i>Bacteroides</i>	+	7.2																
<i>Veillonella</i>		+																
<i>Neisseria</i>			+															
<i>Branhamella</i>				+														
<i>Acinetobacter</i>			7.3		+													
<i>Moraxella</i>						+												
<i>Brucella</i>					7.4	+												
<i>Bordetella</i>						+												
<i>Chromobacterium lividum</i>							+	7.5										
<i>Alcaligenes</i>								+										
<i>Flavobacterium</i>									+	7.6								
<i>Pseudomonas</i>										+								
<i>Actinobacillus</i>											+							
<i>Pasteurella</i>											+	7.7						
<i>Necromonas</i>											+							
<i>Cardiobacterium</i>												+						
<i>Chromobacterium violaceum</i>													+					
<i>Beneckea</i>													+					
<i>Vibrio</i>													+	7.8				
<i>Plesiomonas</i>													+					
<i>Aeromonas</i>													+					
Enterobacteria														+	7.9			
<i>Haemophilus</i>															+			
<i>Eikenella</i>																+		
<i>Campylobacter</i>																	+	
<i>Streptobacillus</i> †																		+
Mycoplasmas																		+

- * No growth in air; growth in air+CO₂.
- † No growth in air or anaerobically; growth in 5-6% O₂.
- ‡ Also *Shigella dysenteriae* 1 (Shiga's bacillus).
- Typical form
- x Not known.

□ Cultural characters of these organisms can be found in tables with the number indicated.

NT Not testable by usual methods. Fermentative.

Catalase Production

The slide method using 3% H₂O₂ was used to indicate catalase activity.

Oxidase Production

A 1% solution of p-aminodimethylaniline oxalate (Difco) was used to test for production of oxidase. The solution was used to flood 48-hour cultures on TSA. Positive colonies became pink, then maroon, and finally black, giving definite results within 3 minutes.

Acid Production from Glucose

Production of acid from glucose was tested using Phenol Red Dextrose Broth (Difco).

Oxidation or Fermentation of Glucose

The glucose OF medium of Hugh and Leifson was inoculated in duplicate. A layer of melted soft paraffin (petrolatum) was added to one of the tubes to a depth of about 1 cm. The tubes were incubated at 20°C and examined for up to 14 days.

G. METHOD FOR DETERMINATION OF FECAL COLIFORMS

MOST PROBABLE NUMBER TECHNIQUE FOR FECAL COLIFORMS (APHA, 1975)

Procedure for Surface Water Samples

0.1, 1.0 and 10.0 mL aliquots of undiluted lake water are aseptically inoculated into 2 sets (5 tubes per set) of single strength (5 mL) and 1 set of double strength lactose broth (10 mL), respectively. After vortexing, all 15 tubes are incubated at $35 \pm 0.5^\circ\text{C}$ for 24 and 48 hrs. Those tubes showing the presence of gas are recorded as positive.

A sterile metal loop with a minimum diameter of 3 mm is used to transfer a loopful of culture from each lactose broth tube (both positive and negative) to E. coli broth (EC) tubes. When making the transfers, first gently shake or mix the lactose tubes by rotating. Inoculated tubes are placed in a water bath at $44.5 \pm 0.2^\circ\text{C}$ for 24 ± 2 hr. The EC tubes should be put in the water bath within 30 minutes after inoculating. The depth of water in the bath should be sufficient to immerse the tubes to the upper level of the medium.

MPN tables are used to calculate the most probable number of fecal coliforms per 100 mL.

- NOTE:
1. The portions of the water sample used for inoculating the lactose broth fermentation tubes may vary in size and number depending upon the sample, but in general should be decimal multiples and submultiples of 1 mL.
 2. If gas is formed as a result of fermentation, the broth medium will become cloudy.
 3. Gas production in a fermentation tube within 24 hrs. or less is considered a positive reaction indicating fecal origin. Failure to produce gas (growth sometimes occurs)

constitutes a negative reaction indicating a source other than the intestinal tract of warm-blooded animals.

Procedure for Sediment Samples

Using aseptic technique, 0.2 mL of the sediment supernatant (see Preparation of Sediment for Analysis Section) is inoculated into each of 5 tubes of single strength lactose broth (5 mL). 2.0 mL of the supernatant is inoculated into a set of double strength lactose broth tubes (10 mL). All 15 tubes are then vortexed and incubated for 24 and 48 hrs. at $35 \pm 0.5^{\circ}\text{C}$. Those tubes showing gas fermentation are recorded as positive.

A loopful of culture from each lactose broth tube (both positive and negative) is transferred to EC broth and incubated for 24 hrs. at $44.5 \pm 0.2^{\circ}\text{C}$. All tubes showing gas formation are scored as positive. The most probable number of coliforms per 100 mL is then determined from MPN tables.

MEMBRANE FILTRATION METHOD FOR FECAL COLIFORMS (DUTKA, 1978)

The membrane filter procedure utilizes an enriched lactose medium, m FC agar, that depends upon an incubation temperature of $44.5 \pm 0.2^{\circ}\text{C}$ for its selectivity. Test batches of medium for satisfactory productivity by preparing dilutions of a culture of E. coli and filtering appropriate volumes to give 20 to 80 colonies per filter. Verify enough colonies, obtained from natural samples, to establish the absence of false positives. Tight-fitting plastic Petri dishes are necessary for the procedure because the membrane filter cultures must be submerged in a water bath during incubation.

The lake water sample is shaken vigorously, and varying dilutions are prepared depending upon the appearance of the sample. The amount of lake water usually used is in the range of 5, 10, 15, 25, 50 or 100 mL, and it is placed into a sterile 100 mL graduated cylinder. Sterile, buffered water is added to the cylinder until the total amount of lake water or lake water plus sterile buffered water amounts to 100 mLs. The sample is then passed through a membrane filter with a pore size of $.45 \mu\text{m}$, (Gelman GN-6 filters). Once the water sample has been filtered, the membrane is aseptically placed onto a m FC plate without allowing air to be trapped between the filter and the surface of the agar. Plates containing filters are placed in a Whirlpack bag (water tight bag), sealed, inverted and anchored below the water surface in the 44.5°C water bath to maintain critical temperature requirements. Incubation is for 4 hours at 35°C followed by 18-20 hours at $44.5 \pm 0.2^{\circ}\text{C}$. After the incubation period, the plates are examined under a stereoscopic microscope (10x magnification) for the growth of blue colonies. These colonies are counted to determine the amount of fecal coliforms in the lake water sample. Nonfecal coliform colonies are gray to cream coloured.

MEMBRANE FILTER TECHNIQUE FOR FECAL COLIFORMS (PAGEL and VLASSOFF, 1979)

Perform filtrations of water samples and place the membrane filter on m-TEC medium in 50 x 9 mm disposable Petri dishes. When a sufficient number of filtrations have been performed (during filtration plates may be maintained at room temperature for up to 2 hours if necessary), place 15-30^(a) Petri dishes containing their membrane filters in a plastic cakette (32 x 22 x 9.5 cm) supplied with 100 mL ice (in two 50 mL aliquots each contained in 8 oz. Pharal jars). The ice is prepared by dispensing 50 mL of tap water into these jars, and maintaining them overnight at -4°C. The two ice jars should be placed at each end of the cakette with the Petri dishes (stack of 2-3 plates) in the centre (preferably place plates at a distance of 1-2 cm from the ice jars).

Incubate each cakette (with ice and Petri dishes) at 44.5°C for 23 ± 1 hour. Do not open/disturb the cakette or introduce additional plates once the incubation of a particular cakette has started. Label each cakette properly to indicate the time of incubation and the time of counting colonies on the membrane. The fecal coliforms should be counted immediately after removing plates from the incubator.

H. METHOD FOR DETERMINATION OF FECAL STREPTOCOCCI

MEMBRANE FILTER TECHNIQUE FOR FECAL STREPTOCOCCI (APHA, 1971)

Procedure for Surface Water Samples

Membrane filtration is used to enumerate the enterococci in lake water samples. Prior to filtration, each lake water sample is vigorously shaken. 10, 25 and 50 mL volumes of lake water are diluted with cold sterile, buffered water to obtain a final volume of 100 mL. An undiluted 100 mL sample of lake water is also prepared. Each prepared water sample is then filtered through a Gelman GN-6 (pore size 0.45 μm) filter, pre-wet with sterile, buffered water. After each filtration, the filter apparatus is rinsed with sterile, buffered water and the filter aseptically transferred to Petri dishes of m Enterococcus Agar (Difco). The filter is carefully placed on the agar to ensure that there are no air bubbles trapped underneath. The Petri dishes are allowed to stand for 30 minutes before incubating at $35 \pm 0.5^\circ\text{C}$ for 48 hrs. Using stereoscopic microscope (10x), all light and dark red colonies are counted as enterococci.

(a) For the incubation of fewer plates, add some plates for a total of at least 15-20 plates per cakette

MEMBRANE FILTER TECHNIQUE FOR FECAL STREPTOCOCCI (APHA, 1975)

Procedure for Surface Water Samples

Filter samples of water, or appropriate dilutions prepared in sterile, buffered water, through GN-6 Gelman filters to obtain 20 to 100 colonies on the membrane surface. This colony density range is greater than the 20 to 80 total coliform range because of the increased selectivity of fecal streptococci media. Transfer the membrane filter directly onto KF Streptococcus agar, avoiding air bubbles. Incubate the plates in a well humidified incubator for 48 ± 3 hrs. at $35 \pm 0.5^{\circ}\text{C}$. Using a stereoscopic microscope (10x) count all dark red to pink colonies as streptococci.

Confirmed Test

Pick a typical colony from the membrane filter and inoculate onto brain heart infusion agar. Incubate at 35°C for 24 to 48 hrs. Using a loopful of growth from the brain heart infusion agar make a smear and add a few drops of 3% hydrogen peroxide. The absence of bubbles, constituting a negative catalase test, is indicative of streptococci.

Transfer a loopful of the brain heart infusion agar culture into brain heart infusion broth and incubate at 45°C for 48 hrs. Transfer another loopful of growth into bile broth medium (40 mL sterile 10% oxgall solution added to 60 mL sterile brain heart infusion broth) and incubate at 35°C for 3 days. Growth in the above two media is a positive test for fecal streptococci.

Procedure for Sediment Samples

Weigh out 10 gm, 1 gm or 0.1 gm of sediment or prepared sediment supernatant (see Preparation of Sediment for Analysis Section) and use 20 mL, 2 mL and 0.2 mL. Use 10 mL single-strength azide dextrose broth for inocula of 1 mL or 1 gm or less and double-strength broth for larger sized inocula. Inoculate the series of 15 MPN tubes and incubate at $35 \pm 0.5^{\circ}\text{C}$. Examine each tube for the presence of turbidity at the end of 24 ± 2 hrs. or 48 ± 3 hrs.

All azide dextrose broth tubes showing turbidity after 24 or 48 hrs. should be treated as follows. Transfer three loopfuls of growth from each azide dextrose broth tube to a tube containing 10 mL ethyl violet azide broth. Incubate the inoculated tubes for 24 hrs. at $35 \pm 0.5^{\circ}\text{C}$. A positive test for fecal streptococci is indicated by the formation of a purple button at the bottom of the tube, or occasionally by dense turbidity. Record all positive results and discard the tubes. If no growth occurs in ethyl violet azide (EVA) broth in 24 hrs., reinoculate the tubes with an additional three loopfuls from the original positive azide culture and incubate for an additional 24 hrs.

I. METHOD FOR DETERMINATION OF STAPHYLOCOCCUS AUREUS

MEMBRANE FILTRATION METHOD FOR ISOLATION OF *Staphylococcus aureus*

Procedure for Lake Water Samples

The medium used for this isolation is Difco Vogel-Johnson agar^(b) and the method is one of membrane filtration. The Gelman GN-6 filter is used, with a pore size of .45 μm . The filter is aseptically placed on the filter holder. It is wet with sterile, buffered water for better adhesion to the filter grid. The slow addition of 100 mL of a combination of lake water and sterile, buffered water proceeds next. The range of sample dilutions used is 25, 50 and 100 mL; depending on the appearance of the sample.

A 25 mL volume of lake water is measured out in a 100 mL graduated cylinder and then the remainder of the cylinder is filled with sterile, buffered water to the 100 mL mark. This same procedure is followed for the 50 and 100 mL lake water dilutions. Following the addition of the sample, the filter is rinsed with sterile, buffered water. It is aseptically removed and placed on a Vogel-Johnson agar plate. The filter is carefully laid down on the plate surface to avoid trapping air bubbles, for the bubble elevations cause changes in atmospheric pressure on the agar surface, which does not allow proper contact with the nutrients below, and hence growth would be impeded. The plate with the filter is allowed to stand for 30 minutes at room temperature before incubation at 37°C for 48 hours. After incubation, the plate is examined using a stereoscopic microscope and magnification of 10x; the colonies observed and counted are of the black, round, and shiny type. Confirmation for *Staphylococcus aureus* proceeds by picking six of the above typical colonies from the Vogel-Johnson plate and streaking on Mannitol Salts agar plates. These plates are incubated for 24 hours at 37°C; colonies which grow out with yellow zones or peripheries surrounding them are picked and subjected to Gram's staining and coagulase testing for confirmation of *Staphylococcus aureus*.

Procedure for Sediment Samples

The primary method for isolation of *Staphylococcus aureus* from sediment is by inoculation into m *Staphylococcus* broth. 10 grams (wet weight) of the sediment sample is weighed out on a Mettler balance and added to 100 mL of m *Staphylococcus* broth, contained in an Erlenmeyer flask with glass beads. The flask is shaken well and incubated at 37°C for 24 hours. Dilutions of 10^{-2} , 10^{-3} and 10^{-4} are made by serial transferrals into sterile, buffered water from the 10^{-1} flask. 0.1 mL amounts of the dilutions are spread over the surface of Vogel-Johnson agar plates and incubated again for 24 hours at 37°C. The confirmatory procedure is the same as for lake water above.

(b) With 0.5% sodium pyruvate added (R. Alico, personal communication)

Pre-enrichment Procedure for Lake Water Samples

The flask containing the water sample is shaken vigorously 5 to 10 times, and a 10 mL aliquot of the sample is pipetted into 90 mL of m Staphylococcus broth contained in an Erlenmeyer flask, to which glass beads have been added. The glass beads increase the surface area of the medium and in so doing aid in dissolving or distributing the water sample inoculum evenly throughout the broth. This constitutes a 10^{-1} dilution. This mixture is allowed to incubate for 24 hours at 37°C , to have a culture of even, turbid constitution. Incubation for longer than 24 hours results in the formation of a pellicle, which is undesirable, since well distributed aliquots of the bacteria present cannot be withdrawn to make further dilutions. Thus after 24 hours of incubation, 10^{-2} and 10^{-3} are made, by shaking the 10^{-1} flask, withdrawing 1.0 mL and transferring it to 9.0 mL of sterile, buffered water, contained in a test tube, this represents the 10^{-2} dilution. The same process of transferral is followed for producing the 10^{-3} dilution as for the 10^{-2} dilution. 0.1 mL aliquots of these 10^{-1} , 10^{-2} and 10^{-3} dilutions are spread over the surface of Vogel-Johnson agar plates using a bent glass Drygalski rod. These plates are allowed to incubate for 24 hours at 37°C , after which the total number of black, shiny and round (Staphylococcus aureus) colonies are counted on each plate for each dilution. Six typical black colonies from each plate are picked and transferred to Mannitol Salts agar plates.

ADDITIONAL STAPHYLOCOCCAL TESTS

Staphylococcal isolates from Vogel-Johnson agar plates were subjected to the following tests:

- (a) Coagulase test using Coagulase Rabbit Plasma (Difco)
- (b) Coagulase test using Coagulase Rabbit Plasma EDTA (Difco)
- (c) Gram stain
- (d) Reaction on Mannitol Salt agar (Difco)
- (e) Hemolysis on sheep blood agar
- (f) Production of pigment on TSA (BBL)
- (g) DNase test on DNase Test agar with Methyl Green
- (h) Lipase production on Colbeck EY agar (Difco)
- (i) Thermonuclease test

J. METHOD FOR DETERMINATION OF *Pseudomonas aeruginosa*

MOST PROBABLE NUMBER TECHNIQUE FOR *Pseudomonas aeruginosa* (APHA, 1975)

Procedure for Surface Water Samples

Presumptive Test

Sterile tubes with 10 mL of double-strength asparagine broth (5 tubes in a set) are inoculated with 10 mL of lake water sample. Two sets of tubes containing 10 mL of single-strength broth are inoculated with 1 mL of 0.1 mL of sample. The tubes are then incubated for four days at 41.5°C. At the end of the incubation period the tubes are examined under long-wave ultraviolet light (black light) in a dark room. A positive presumption test is indicated by a greenish fluorescent pigment.

Confirmatory Test

Presumptive positive tubes of asparagine broth are confirmed by transferring 0.1 mL of each positive tube into tubes containing 5 mL of sterile acetamide broth. The inoculated acetamide tubes are incubated at 41.5°C for three days. A positive confirmed test reaction is the development of a high pH which is indicated by a purple colour.

Changes in Method

Presumptive Test

- (a) Tubes are incubated for four days at 41.5°C and are then left at room temperature for two days before results are read.
- (b) Production of a greenish fluorescent pigment, bluish fluorescent pigment or a bluish-green fluorescent pigment constitutes a positive presumptive test.

Confirmatory Test

- (a) Positive and negative tubes of asparagine broth cultures are confirmed for the presence or absence of *Pseudomonas aeruginosa*.
- (b) Other confirmatory tests are also performed. A loopful of each of the asparagine broth cultures is streaked onto Heart Infusion agar plates. The HI plates are incubated for 24 hrs. at 37°C. Isolated colonies from the HI plates are then streaked onto Skim Milk plates and then onto King's A plates. The Skim Milk plates are incubated at 37°C for 2 days. A positive confirmed test is indicated by a zone of clearing around the growing colony. King's A plates are incubated at 37°C for 4 days. A positive confirmed test is indicated by a green pigmentation of the media.

Procedure for Sediment Samples

Presumptive Test

Ten grams (wet weight) of the sediment sample are placed into each of five sterile double-strength asparagine broth tubes. Two sets of tubes containing 10 mL of single-strength broth are inoculated with 1 g and 0.1 g of sediment. (Five tubes constitutes a set). The inoculated tubes are incubated at 41.5°C for four days. At the end of the incubation period the tubes are examined under long wave ultraviolet light (black light) in a dark room. A greenish fluorescent pigment indicates a positive presumptive test.

Confirmatory Test

Presumptive positive tubes of asparagine broth are confirmed by adding 0.1 mL of the culture into tubes containing 5 mL of sterile, acetamide broth. Inoculated acetamide tubes are incubated for three days at 41.5°C. The development of a high pH which is demonstrated by a purple colour, indicates a positive confirmed test reaction.

Changes in Method

Presumptive Test

- (a) After the inoculated asparagine tubes are incubated for four days at 41.5°C they are left at room temperature for another two days for a more definitive pigmentation production.
- (b) Production of a greenish fluorescent pigment, bluish fluorescent pigment or a bluish-green fluorescent pigment constitutes a positive presumptive test.

Confirmatory Test

- (a) Both positive and negative tubes of asparagine broth cultures are confirmed for the presence or absence of Pseudomonas aeruginosa by inoculating 0.1 mL of the culture into sterile acetamide tubes.
- (b) Other confirmatory tests are also performed. A loopful of each of the asparagine broth cultures is streaked onto Heart Infusion Agar plates. After a 24 hr. incubation at 37°C, isolated colonies are streaked onto Skim Milk plates and King's A plates. The Skim Milk plates are incubated at 37°C for two days. A positive confirmatory test is indicated by a zone of clearing around the growing colony. King's A plates are incubated for four days at 37°C. A positive confirmatory test is indicated by green pigmentation of the media around the growing colony.

MEMBRANE FILTRATION TECHNIQUE FOR *Pseudomonas aeruginosa* (APHA, 1975)

Procedure for Surface Water Samples

Collection of Samples

Pre-sterilized glass bottles are used as sample containers. The minimum volume of sample required is 150 mL or 6 ozs. Samples are collected approximately a 1/2 metre below the surface of the lake and placed in coolers containing ice packs. *Pseudomonas aeruginosa* levels will undergo change even upon refrigeration, therefore, immediate delivery to the lab is essential. Analyses must be done as soon as possible, within 6 hrs., but not exceeding 24 hours of collection.

Field Sample Analysis and Culture Medium

Samples are diluted with sterile, buffered water to obtain a total volume of 100 mL. Dilutions are made with a graduated cylinder and are as follows:

	Volume of Sample (mL)	Volume of Sterile Buffered Water (mL)	Total Volume (mL)
1)	5	95	100
2)	10	90	100
3)	25	75	100
4)	50	50	100
5)	100	0	100

The number of dilutions prepared per sample is dependent upon the appearance of the water. More dilutions are made if the sample is murky.

Using aseptic technique, membrane filters (Gelman GN-6, 0.45 μm) are placed on the filter holder. The membrane is pre-wet with sterile, buffered water. The 100 mL of diluted sample is slowly added. After filtration through the membrane, approximately 10 mL of sterile buffered water is poured through as a rinse procedure. The membrane is then transferred aseptically to mPA, mPA-B or mPA-C agar plates. The entire bottom surface of the membrane must be flush with the surface of the agar. No bubbles may be present between the agar and the filter. It is recommended to allow the plates to stand 30 minutes before incubation at 41.5°C. mPA plates are incubated for 4 days, mPA-B plates for 2 days and mPA-C for 24 hours.

After incubation, the plates are examined using a stereoscopic microscope (10x) or a Quebec colony counter. All flat colonies with a light outer rim and brownish to greenish-black centre are counted as Pseudomonas aeruginosa. Result are reported as number of organisms per 100 mL of sample.

REFERENCES

- American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed. American Public Health Association, Inc., New York.
- American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater, 14th ed. American Public Health Association, Inc., New York.
- Cowan, S.T. and D.J. Steel. 1974. Manual for the identification of medical bacteria. Cambridge University Press.
- Dutka, B.J. (Editor). 1978. Methods for Microbiological Analysis of Waters, Wastewaters and Sediments. Inland Waters Directorate, Scientific Operations Division, Canada Centre for Inland Waters, Burlington, Ontario, Canada.
- Gerba, C.P., E.M. Smith, G.E. Schaiberger, and T.D. Edmond. 1979. Field evaluation of methods for the detection of enteric viruses in marine sediments. Methodology for Biomass Determinations and Microbial Activities in Sediments. ASTM Special Technical Publication 673, Litchfield and Seyfried eds. :64-74.
- King, E.O., M.K., Ward, and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301.
- Pagel, J.E. and L.T. Vlassoff. 1979. Determination of performance characteristics for fecal coliform enumeration procedures. Abstracts of the Annual Meeting of the American Society for Microbiology: 229.
- Young, M. 1978. Outline of Microbiological Methods. Ontario Ministry of the Environment Report: 39.

APPENDIX E

MEDIA PREPARATION

Table of Contents

	<u>Page</u>
1. STOCK PHOSPHATE BUFFER SOLUTION.....	147
2. HEART INFUSION AGAR	147
3. TRYPTICASE SOY BROTH (TSB) PLUS AGAR	148
4. TRYPTICASE SOY AGAR (TSA)	148
5. NUTRIENT AGAR 1.5%.....	148
6. CASEIN-PEPTONE-STARCH (CPS) AGAR.....	149
7. LACTOSE BROTH	149
8. <u>E. coli</u> (EC) BROTH	150
9. mFC AGAR	150
10. m STAPHYLOCOCCUS BROTH.....	151
11. m-TEC AGAR	151
12. KF STREPTOCOCCUS AGAR	152
13. m ENTEROCOCCUS AGAR	152
14. AZIDE DEXTROSE BROTH.....	153
15. ETHYL VIOLET AZIDE (EVA) BROTH	153
16. VOGEL-JOHNSON (VJ) AGAR	153
17. ASPARAGINE BROTH.....	154
18. ACETAMIDE BROTH.....	154
19. SKIM MILK AGAR (BROWN AND SCOTT FOSTER MODIFICATION)	155
20. KING'S A MEDIUM (KING, WARD AND RANEY, 1954).....	155
21. MODIFIED DRAKE'S MEDIUM (ONTARIO MINISTRY OF THE ENVIRONMENT)	156
22. PSEUDOSSEL AGAR	156
23. mPA, mPA-B AND mPA-C MEDIA	157

1. STOCK PHOSPHATE BUFFER SOLUTION

Dissolve 34.0 g KH_2PO_4 in 500 mL distilled water, adjust to pH 7.2 with 1 N NaOH and dilute to 1000 mL with distilled water.

Preparation of Phosphate Buffered Water

Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium sulphate (50 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ /L distilled water) to 1 litre of distilled water. Dispense as 90 or 9.0 mL dilution blanks or as 1 litre rinse water aliquots. Sterilize by autoclaving for 20 minutes at 15 psi.

2. HEART INFUSION AGAR

Beef heart infusion	500 g
Tryptose	10 g
Sodium chloride	5 g
Bacto-agar	15 g

Preparation of Heart Infusion Agar

Suspend 40 g of Bacto-heart Infusion agar in 1000 mL of cold distilled water and heat to boiling to dissolve the medium completely. Sterilize by autoclaving for 15 minutes at 15 psi. (121°C). The final pH of the medium will be 7.4.

Modification: A 100 ppm concentration of Actidione is incorporated in the medium by adding 10 mL of a 1% solution of Actidione (filter sterilized) to HI agar before pouring the plates.

Preparation of Heart Infusion (HI) Broth Plus Agar

Add 25 g of Difco Bacto-heart Infusion Broth to 1000 mL distilled water. 20 g of Difco Bacto-agar is added as a solidifying agent. Dissolve completely and sterilize in an autoclave for 15 minutes at 15 psi. Dispense 25-30 mL of sterile media into Petri plates and allow to solidify.

Modification: As of October 22, 1979 all HI Agar prepared had 10 mL of a 1% solution of Actidione (100 ppm) added before pouring, (10 mL/L of HI).

3. TRYPTICASE SOY BROTH (TSB) PLUS AGAR

Trypticase peptone	17 g
Phytone peptone	3 g
Sodium chloride	5 g
Dipotassium phosphate	2.5 g
Dextrose	2.5 g

Preparation of Trypticase Soy Broth Plus Agar

Dissolve 30 g of BBL Trypticase Soy Broth in 1 litre of distilled water. Add 20 g of Difco Bacto-agar as a solidifying agent. Heat to boiling to dissolve the agar completely. Autoclave at 15 psi. for 15 minutes. Cool and pour into Petri plates. Slants can be made by dispensing 5 mL of the heated medium into 16 mm screw cap tubes. Caps must be loose for autoclaving because evaporation of the condensate must be possible. Autoclave and slant after sterilization but before solidification.

4. TRYPTICASE SOY AGAR (TSA)

Trypticase peptone	15 g
Phytone peptone	5 g
Sodium chloride	5 g
Agar	15 g

Preparation of Trypticase Soy Agar

Add 40 g of BBL Trypticase Soy Agar to 1000 mL distilled water. Mix well, agitate while heating, and boil for one minute. Sterilize by autoclaving at 121°C for 15 minutes at 15 psi.

5. NUTRIENT AGAR 1.5%

Beef extract	3 g
Peptone	5 g
Sodium chloride	8 g
Bacto-agar	15 g

Preparation of Nutrient Agar 1.5%

Dissolve 31 g of Difco Bacto Nutrient Agar in 1000 mL of distilled water. Heat to boiling to dissolve the agar. Sterilize in an autoclave for 15 minutes at 15 psi. Cool to 50°C and dispense into Petri dishes.

6. CASEIN-PEPTONE-STARCH (CPS) AGAR

K_2HPO_4	0.2 g
$MgSO_4 \cdot 7H_2O$	0.05 g
Ferric chloride solution (0.5 g $FeCl_3$ in 100 mL distilled water)	0.2 mL
Peptone	0.5 g
Soluble casein	0.5 g
Soluble starch	0.5 g
Glycerol	1.0 g
Agar	20.0 g
Distilled water	1000.0 mL

Preparation of CPS Agar

Mix ingredients in distilled water. Heat to boiling to dissolve the agar. Adjust pH to 7.05 before autoclaving. Sterilize in an autoclave for 18 minutes at 15 psi. Cool to 60°C. Add 10 mL of filter sterilized Actidione (100 ppm) per litre of medium. Adjust pH to 7.2 and pour into Petri plates.

7. LACTOSE BROTH

Beef Extract	3 g
Peptone	5 g
Lactose	5 g

Preparation of Lactose Broth for MPN

Dissolve 13 g of Difco Bacto Lactose Broth in 1000 mL of distilled water. Dispense into test tubes with inverted Durham tubes and sterilize in an autoclave for 15 minutes at 15 psi. (121°C).

	Inoculum	Amount of Medium	Size of Tube (mm)	Total Volume	Dehydrated Lactose Broth Required in g/L
SS Lactose	0.1 mL	5 mL	16	5.1 mL	13.0
	1.0 mL	5 mL	18	6.0 mL	13.0
DS Lactose	10.0 mL	10 mL	20	20.0 mL	26.0

Preparation of EC Broth

9. mFC AGAR

Preparation of m FC Agar

Suspend 52 g of Difco Bacto m FC agar in 1000 mL of distilled water and heat to boiling to dissolve the agar. Add 10 mL of a 1% solution of Rosolic acid in 0.2 N NaOH. Continue to heat for 1 minute. Cool to 50°C and pour into Petri dishes.

10. m STAPHYLOCOCCUS BROTH

Tryptone	10 g
Yeast extract	2.5 g
Lactose	2 g
Mannitol	10 g
Dipotassium phosphate	5 g
Sodium chloride	75 g

Preparation of m Staphylococcus Broth

To 1000 mL of cold distilled water add 104 g of Difco Bacto m Staphylococcus broth and warm to dissolve completely. To 250 mL Erlenmeyer flasks add 95 mL of the Staph. broth and 10-15 glass beads. Sterilize for 15 minutes at 15 psi. (121°C).

11. m-TEC AGAR

m-TEC Agar is used for the detection and isolation of thermotolerant E. coli.

Proteose Peptone No. 3 (Difco)	5.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sodium chloride	7.5 g
K ₂ HPO ₄	3.3 g
KH ₂ PO ₄	1.0 g
Sodium lauryl sulphate	0.2 g
Sodium desoxycholate	0.1 g
Bromocresol purple	80.0 mg
Bromophenol red	80.0 mg
Agar	15.0 g

Preparation of m-TEC Agar

Mix ingredients into 1 litre of distilled water. Heat to dissolve the agar. Adjust pH to 7.2 before autoclaving. Sterilize in an autoclave for 15 minutes at 15 psi. Allow to cool to 50°C and pour aseptically into Petri plates. Final pH 7.1 ± 0.2.

12. KF STREPTOCOCCUS AGAR

Proteose Peptone No. 3	10 g
Yeast extract	10 g
Sodium chloride	5 g
Sodium glycerophosphate	10 g
Maltose	20 g
Lactose	1 g
Sodium azide	0.4 g
Bromocresol purple	0.015 g
Agar	20 g

Preparation of KF Streptococcus Agar

Suspend 76.4 g in 1000 mL cold distilled water. Heat to boiling to dissolve completely and continue heating for an additional 5 minutes. Do not autoclave since overheating will lower the pH and render the medium less productive. Cool to 50-60°C and add 1 mL Bacto-TTC Solution 1% per 100 mL sterile medium. Mix to obtain uniform distribution of the TTC throughout the medium. Cool to 45°C and pour plates. TTC = 2, 3, 5 - triphenyl tetrazolium chloride.

13. m ENTEROCOCCUS AGAR

Tryptose	20 g
Yeast extract	5 g
Dextrose	2 g
Dipotassium phosphate	4 g
Sodium azide	0.4 g
Bacto-agar	10 g
2,3,5 - Triphenyl tetrazolium chloride	0.1 g

Preparation of m Enterococcus Agar

Suspend 42 g of Difco Bacto m Enterococcus Agar in 1000 mL of cold distilled water and heat to boiling to dissolve the agar completely. Dispense approximately 25-30 mL of dissolved medium into sterile Petri dishes and allow to solidify.

14. AZIDE DEXTROSE BROTH

Beef extract	4.5 g
Tryptose	15.0 g
Dextrose	7.5 g
Sodium chloride	7.5 g
Sodium azide	0.2 g

Preparation of Azide Dextrose Broth

Dissolve 34.7 g in 1000 mL of distilled water. Sterilize in autoclave for 15 minutes at 15 psi. (121°C). If inocula are larger than 1 mL per 10 mL of medium, the medium should be prepared in double or multiple strength.

15. ETHYL VIOLET AZIDE (EVA) BROTH

Tryptose	20 g
Dextrose	5 g
Dipotassium phosphate	2.7 g
Monopotassium phosphate	2.7 g
Sodium chloride	5 g
Sodium azide	0.4 g
Ethyl violet	0.00083 g

Preparation of EVA Broth

Dissolve 35.8 g in 1000 mL of distilled water. Distribute in tubes in 10 mL amounts. Sterilize for 15 minutes at 15 psi. (121°C).

16. VOGEL-JOHNSON (VJ) AGAR

Tryptone	10 g
Yeast extract	5 g
Mannitol	10 g
Dipotassium phosphate	5 g
Lithium chloride	5 g
Glycine	10 g
Bacto-agar	15 g
Phenol red	0.025 g

Preparation of Vogel-Johnson (VJ) Agar with Actidione and Na-Pyruvate

Dissolve 60 g of Difco Bacto VJ agar in 1000 mL cold distilled water. Heat to boiling to dissolve the agar completely. Sterilize in an autoclave for 15 minutes at 15 psi (121°C). Cool to 50-55°C. Then add the following:

1. 20 mL/L of a 1% Bacto-Chapman Tellurite Solution.
- 2.* 10 mL/L Actidione (100 ppm) which has been filter sterilized.
- 3.* Add (filter sterilized) sodium pyruvate to a concentration of 0.5%.

Pour into Petri plates and allow to solidify.

- 2.* 1 g of Actidione (Upjohn) to 100 mL of distilled water. Autoclave for 15 minutes at 15 psi. Add 10 mL of this solution to 1 litre of media.
- 3.* 25 g of Na-pyruvate (BDH) in 100 mL distilled water. Filter sterilize. Add 20 mL of this solution to 1 litre of autoclaved VJ medium. (The addition of Na-pyruvate was suggested by Robert Alico, Department of Biology, Indiana University of Pennsylvania).

17. ASPARAGINE BROTH

Asparagine, DL	3.0 g
Anhydrous K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Distilled water	1.0 L

Preparation of Asparagine Broth

Double strength asparagine broth can be prepared by doubling the ingredients in 1000 mL.

Adjust pH to 6.9 - 7.2. Dispense into 20 mm test tubes and sterilize in an autoclave for 15 minutes at 15 psi (121°C).

18. ACETAMIDE BROTH

Acetamide Broth may be used as a confirmatory test for the presence of Pseudomonas aeruginosa. This medium may not be available in dehydrated form and may be prepared from the following:

Acetamide	10.0 g
Sodium chloride	5.0 g
Anhydrous K_2HPO_4	1.39 g
Anhydrous KH_2PO_2	0.73 g

MgSO ₄ ·7H ₂ O	0.5 g
Phenol red	0.012 g
Distilled water	1 L

Preparation of Acetamide Broth

Adjust pH to 6.9 - 7.2 before sterilization. Dispense 5 mL of broth into 13 mm test tubes. Autoclave for 15 minutes at 15 psi. (121°C).

19. SKIM MILK AGAR (BROWN AND SCOTT FOSTER MODIFICATION)

Skim Milk Solution

Carnation instant non-fat milk	100 g
Distilled water	500 mL

Stir without heat to dissolve for 30 minutes.

Agar Solution

Agar (Bacto)	15 g
Distilled water	500 mL

Heat to boiling (approximately 90°C) for approximately 10 - 12 minutes.

Preparation of Skim Milk Agar

Autoclave solutions separately for 12 minutes at 121°C. When cooled to 50 - 55°C (approximately 30 minutes) add skim milk solution to agar solution. Dispense aseptically into sterile Petri plates.

20. KING'S A MEDIUM (KING, WARD AND RANEY, 1954)

King's A may be used to detect pigment production by Pseudomonas aeruginosa.

It may be prepared from the following:

Peptone	20.0 g
Glycerol	10.0 g
Anhydrous K ₂ SO ₄	10.0 g
Anhydrous MgCl ₂	1.4 g
Agar	15.0 g
Distilled water	1000.0 mL

Preparation of King's A Medium

Dissolve ingredients numbers 1 - 4 in water and adjust to pH 7.2 before adding agar. Add agar and heat gently to dissolve. Autoclave for 15 minutes at 15 psi. Cool and pour into sterile Petri plates.

21. MODIFIED DRAKE'S MEDIUM (ONTARIO MINISTRY OF THE ENVIRONMENT)

L-Asparagine	2 g
Anhydrous dipotassium phosphate	1 g
MgSO ₄ ·7H ₂ O	0.5 g
Anhydrous potassium sulphate	10 g
Glycerol	10 g
Distilled water	1 L

Preparation of Drake's Medium

Dispense into tubes in 4 mL amounts. Double strength Drake's medium is used for 10 mL tubes. Autoclave at 15 psi for 15 minutes. Final pH 7.6 ± 0.2.

22. PSEUDOSEL AGAR

Gelysate Peptone	20 g
Magnesium chloride	1.4 g
Potassium sulfate	10 g
Agar	13.6 g
Cetrimide	0.3 g

Preparation of Pseudosel Agar

Suspend 45.3 g of the powder in a litre of distilled water. Add ten mL of glycerol. Heat with frequent agitation and boil for one minute. Dispense and sterilize by autoclaving at 118 to 121°C for 15 minutes. Final pH 7.2 ± 0.2.

23. mPA, mPA-B, mPA-C MEDIA

<u>Component per litre</u>	<u>mPA</u>	<u>mPA-B</u>	<u>mPA-C</u>
L-lysine hydrochloride	5.0 g	5.0 g	5.0 g
Sodium chloride	5.0 g	5.0 g	5.0 g
Yeast extract	2.0 g	2.0 g	2.0 g
Xylose	2.5 g	1.25 g	1.25 g
Sodium thiosulphate	6.8 g	5.0 g	5.0 g
Sucrose	1.25 g	1.25 g	1.25 g
Lactose	1.25 g	1.25 g	1.25 g
Phenol red	0.08 g	0.08 g	0.08 g
MgSO ₄ .7H ₂ O	-	1.5 g	1.5 g
Ferric ammonium citrate	0.8 g	0.8 g	0.8 g
Oxid agar number 4	15.0 g	15.0 g	12.0 g
Distilled water	1000 mL	1000 mL	1000 mL

mPA: Adjust pH to 6.5 and sterilize for 15 minutes. Cool to 50 - 55°C; carefully readjust pH to 7.1 ± 0.1 and add the following dry antibiotics/litre of agar base:

Sulfapyridine	176 mg
Kanamycin	8.5 mg
Nalidixic acid	37 mg
Actidione	150 mg

After mixing dispense in 3 mL quantities in 50 x 12 mm Petri plates. Poured plates may be stored at 2 - 10°C for 1 month.

mPA-B: Mix ingredients and let stand 10-15 minutes, mix well and autoclave for 20 minutes at 121°C. Cool to 55°C in a waterbath and add the following antibiotics as a dry powder:

Sulfapyridine	176 mg
Kanamycin	8.5 mg
Nalidixic acid	37 mg
Actidione	150 mg

Adjust pH to 7.1 ± 0.1. Dispense in 4 - 7 mL quantities into small Petri dishes. Plates may be stored up to 2 weeks at 2 - 6°C.

mPA-C: The basal medium is dissolved by boiling and the pH is adjusted to 7.2. The medium is cooled to 55°C before adding the antibiotics. The antibiotics are prepared as liquid concentrates which are frozen until needed. Antibiotics are added to mPA-C to provide a final concentration of (µg/mL): kanamycin 8 and nalidixic acid 37. The medium is then poured into 50 mm Petri dishes.

ACKNOWLEDGEMENTS

This study was carried out under contract by the University of Toronto. Professor P.L. Seyfried was the principal investigator. Dr. R.S. Tobin of the Bureau of Chemical Hazards, Department of National Health and Welfare was the contract scientific authority. Virological studies were performed by Ms. C.L. Cherwinsky and Dr. G. Jenkins, Microbiology Section, Ontario Ministry of the Environment. Pathogenic amoebae analyses were performed by Professor D. Cotter, University of Windsor. The assistance of all members of the Advisory Group in the design and review of the progress of this study is gratefully acknowledged.

MEMBERS OF THE
ADVISORY GROUP TO THE EPIDEMIOLOGICAL
STUDY OF THE GREAT LAKES BEACHES

Dr. J. Davies
Director, Bureau of Epidemiology
Laboratory Centre for Disease Control
Health and Welfare Canada
Ottawa, Ontario
K1A 0L2

Dr. A. Dufour
U.S. Environmental Protection Agency
5 Harrison Avenue
Wakefield, Rhode Island 02879
U.S.A.

Mr. B.J. Dutka
Head, Microbiology Section
National Water Research Institute
Burlington, Ontario
L7R 4A6

Dr. W.H. LeRiche
Department of Epidemiology
Faculty of Medicine
University of Toronto
Toronto, Ontario
M5S 1A1

Professor P.L. Seyfried
Department of Microbiology and Parasitology
Faculty of Medicine
University of Toronto
Toronto, Ontario
M5S 1A1

Dr. R.S. Tobin (Chairman)
Bureau of Chemical Hazards
Health Protection Branch
Health and Welfare Canada
Ottawa, Ontario
K1A 0L2

Mr. L. Vlassoff
Manager, Microbiology Section
Ontario Ministry of the Environment
Toronto, Ontario
M9W 5L1

EARTH SCIENCES
LIBRARY

